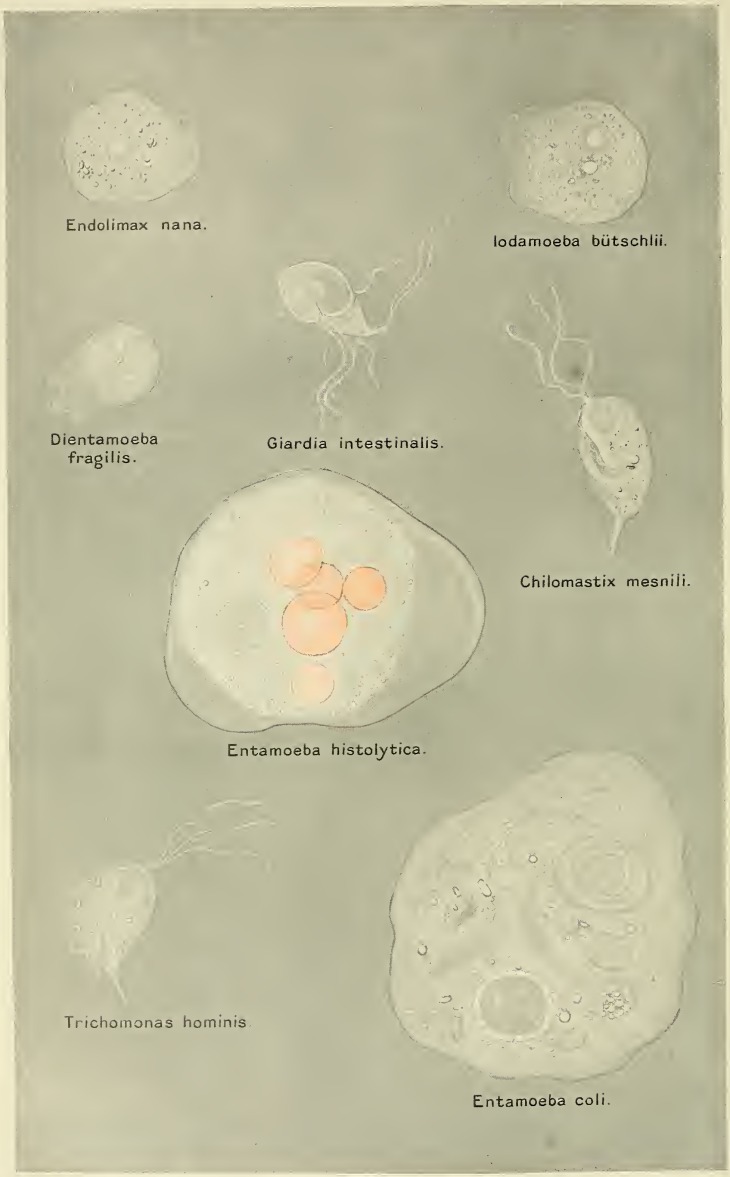
TIMOTHY LEARY

PLATE I.



× 2,000

Some of the Intestinal Protozoa of Man, as they appear when alive and active.

THE INTESTINAL PROTOZOA OF MAN

BY

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AND

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"*O, wonder!*
"*How many goodly creatures are there here*
"*How beauteous mankind is! O brave new world,*
"*That has such people in't!*"
—SHAKESPEARE, *Tempest, V. 1.*

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To our mutual friend

Charles Morley Wenyon

PREFACE.

THE following treatise is addressed to all Zoologists and Medical Men who are interested in the Intestinal Protozoa of Man, but more especially to those whose professional duties demand an intimate practical knowledge of these organisms. During the recent Great War the need for such a work became urgent: and although the War is now ended, and interest in the subject has waned, there must still be many workers, especially in the tropics, to whom a work of this character would be—if properly executed—of very great service.

It appeared to the present authors that such a book—touching upon the two fields of Zoology and Medicine—ought to be written jointly by a zoologist and a medical man: for by such collaboration many mistakes, due to the limited knowledge of either, might obviously be avoided. This consideration, and a mutual interest in the subject, prompted the authors of the following work—Captain O'Connor and myself—to enter into partnership. It was originally agreed between us that we should write the book together, though one of us should be specially responsible for the medical parts, the other for those parts which were purely protozoological.

Unfortunately, it proved impossible to carry out our original intentions. The work was first planned at the end of 1918: but in the autumn of the following year, when the book had only been sketched out and begun, Captain O'Connor left England on a scientific expedition to the Gilbert and Ellice Islands. Further collaboration thus became impossible, and the completion of the work consequently devolved entirely upon me. As many papers on this subject have been published recently, and as I have continued my own researches during the last few years, it will be understood that the book, as it now appears, is in many ways very different from that originally planned.

For several sections of the book I am solely responsible. The most important of these are: the Introduction (Chap. I); the section dealing

with the coprozoic organisms—based largely upon hitherto unpublished researches ; the lists of synonyms, and keys for the determination of genera and species, together with all discussions of systematics and classification ; the references at the end of the volume, and the general bibliographic work throughout. I have also drawn all the illustrations, with the exception of figs. 97-102 (Pl. VI), which I have merely redrawn from Captain O'Connor's originals—these having proved unsuitable for reproduction. Footnotes which contain my personal opinions are distinguished by bearing my initials, whenever it has seemed desirable or necessary to indicate their authorship.

I have thought it right to narrate these particulars here. But my object in so doing is not that I may claim the greater share of credit—if any there be—for our joint performance, but to exonerate my partner from blame for the mistakes which have doubtless been made. During the last eighteen months, whilst I have been engaged in writing and revising the book, and in passing it through the press, I have been entirely deprived of his counsel. I have been unable to discuss with him any of the new work which has appeared. I have changed my views on various subjects as I have learned new facts, and I have had no means of ascertaining whether his views have undergone corresponding changes. Consequently, although Captain O'Connor permitted me—in fact besought me—to make any alterations which appeared to me necessary during the progress of the work, I feel that I have been compelled to take far greater liberties with his contributions than any ordinary collaborator would have a right to take. And while it is my hope that I have not, in the following pages, expressed any views from which Captain O'Connor would dissent, yet I feel it incumbent upon me to point out here that, for any mistakes which have been made, a far greater share of responsibility lies upon my shoulders than upon his.

During the preparation of this work I have fortunately been able to consult Captain S. R. Douglas, I.M.S. (ret.), on medical matters outside my competence. He has also had the kindness to read through Chapters III, VII, and VIII, which have profited by his help and criticism. For these services we offer him here our sincere thanks. I wish also to thank Professor W. Bulloch, F.R.S., for supplying me with a number of references to works which I should else have overlooked. We are further indebted to the Editor of *Parasitology*, Professor G. H. F. Nuttall, F.R.S., for permission to republish fig. 28 (Pl. III) ; and to Lieutenant-Colonel W. Byam, R.A.M.C., and the Oxford University Press, for allowing us to use figs. 27 (Pl. III) and 109-111 (Pl. VII),

which were drawn originally for Byam and Archibald's forthcoming treatise on *The Practice of Medicine in the Tropics.*

I would point out here that the figures have all been drawn—unless the contrary is expressly noted—from actual specimens, with the aid of the camera lucida. They are not diagrammatic. But the figures on the Frontispiece (Pl. I), though not intended to appear schematic, were drawn from memory and imagination. They are composite pictures—made accurately to scale, and as correct as possible in their details, but not copied from any particular specimens. It is impossible to draw an actively moving protozoon with the camera lucida; and the artist who professes to depict such an organism "from life" must always, in reality, first observe it accurately, and then make his drawing from memory—combining the thousands of changing images which have fallen upon his retina into a single fixed and lifeless picture. The figures on Plate VIII are frankly "diagrams" of the same sort, so drawn for a special purpose. They are attempts to show to others, as accurately as is possible by means of single images, the appearances which I have seen upon innumerable occasions. Not one of these figures has been copied from any particular specimen, but each is a general description—with the brush instead of the pen—of the thousands of similar individual objects which have passed before my eyes. It is really impossible to convey an exact impression of such objects by means of drawings; and when such drawings have been more or less effectively executed, it is almost impossible to overcome the difficulties involved in the process of reproduction. The methods by which I have, in the present case, "faked" the figures into a semblance of reality, are too obvious to require comment.

It has been our aim, throughout this work, to be as brief and accurate as possible. We have made no attempt to treat the subject in an encyclopaedic manner, but have aimed rather at producing a practical handbook—a book which will help the beginner, and at the same time assist more serious students in the prosecution of their studies. A work of this character would be of little use if it did not contain full and accurate references, and I have therefore devoted special attention to the bibliographic aspects of the subject. Every work cited has been consulted in the original, and every effort has been made to insure accuracy in quotations and references. Those who have any knowledge of the subject, and who are acquainted with the almost endless bibliographic errors in most works dealing with it, will realize the toil which this has entailed. The references represent,

indeed, the intermittent labour of many years: but I think the time taken over them has not been mis-spent, for it has enabled us to avoid the repetition of many text-book traditions of the unfounded but long-lived type familiar to all students of scientific literature.

It has not been possible to take notice of many works which have appeared in the last few months, but an attempt has been made to incorporate at least a reference to every work of importance which has come to my notice up to the time of going to press. No effort has been made, however, to cite every work that has been written on the subject, since this would have made our references run into many thousands. Hundreds of references have, indeed, been weeded out in the final revision. Judicious selection, rather than compendious collection, has been aimed at in this respect.

It seems to me that it is the duty of every scientific worker to study and weigh what his predecessors and contemporaries have written, and that he should be as careful in quoting them as he is in making and recording his own observations. To neglect to notice the work of others, or to misquote it, is often something more than incivility: it easily leads an author to claim—or to appear to claim—as his own a discovery or observation to which he has no title. But in dealing with the works of others one must constantly note their errors—no less than their good parts. To summarize without criticizing is not possible in a work which aims at being scientific. Error and truth cannot be added together. Consequently, criticism also is a duty to every collector of facts. I have often been taken to task, by reviewers of my previous publications, for the "severity" of my criticisms of the work of others. I wish, therefore, to make this explanation. All my criticisms are directed against opinions or interpretations—not against persons. If a statement is true, it will withstand the severest criticism. If, on the other hand, it is false, it cannot be too severely condemned. I thus see no reason to reproach myself for the severity of any criticisms which I may have made, unless they have unwittingly been unjust to persons or unjustified in matters of fact.

But it is easy to destroy and hard to build, and I would therefore end with the words of the ingenious Dr. Edward Tyson,* who long ago excused himself to perfection upon a like occasion: "My *design* here," said he (and it is ours also), "is not the raising of any *Hypothesis*,

* See his once celebrated memoir on the Tape-worm, *Phil. Trans. Roy. Soc.*, 1683. No. 146.

but the enquiring into the truth of those of *others*. It being much easier to spy others faults, then to avoid them our selvs. In what I have said I have done the former; but can no ways secure my self as to the latter. But in the whole, if I have not hit the mark; I have fairly aimed for it, and it may be some help, and direction to others in the prosecution of this subject."

CLIFFORD DOBELL.

London,
April, 1921.

"È ben più facile insegnare una verità,
che stabilirla sopra le rovine di un errore;
è ben più facile l'aggiungere che il sostituire."

—LEOPARDI.

CONTENTS.

"Reade not to Contradict, and Confute;
Nor to Beleeve and Take for granted;
Nor to Finde Talke and Discourse;
But to weigh and Consider."

—BACON, *Of Studies* (ed. 1625).

THE INTESTINAL PROTOZOA OF MAN.

CHAPTER I.

INTRODUCTION. THE INTESTINAL PROTOZOA OF MAN.

TO speak of Man as a Microcosm—"an abstract or model of the world," as Bacon has it—is an ancient and familiar figure of speech. But the modern scientific writer can hardly stop short at this metaphor: he knows that, within this microcosm, there is a less poetic and still smaller world which has been revealed to the inquiring eye of the microscopist. Man's body is, indeed, itself a macrocosm for innumerable micro-organisms; and it is to one of the microscopic communities inhabiting one small province of this very little world—the Protozoa living in the intestine of Man—that the following treatise is devoted.

The object of this first chapter is to introduce the Intestinal Protozoa of Man to the reader. In the following chapters he will have an opportunity of cultivating their acquaintance more closely; but this acquaintance cannot ripen into intimacy unless he combines the perusal of this book with a study of the organisms themselves.

HISTORIC NOTE.—In the year 1681, Antony van Leeuwenhoek, the illustrious Hollander who discovered the Protozoa and is rightly regarded as the Father of Protozoology, described a "little creature" which he had observed, with the aid of magnifying glasses, in his own stools.* This little creature was the flagellate protozoon now known as *Giardia intestinalis*, and its discovery marks the beginning of our knowledge of the Intestinal Protozoa of Man.

The discovery excited but a passing interest, and lay almost forgotten for over a century and a half. Then, in the year 1854, two

* See Dobell (1920), where these observations are considered in detail.

similar little animals were found in human stools by the French parasitologist Davaine, who subsequently named them "*Cercomonas hominis A*" and "*C. hominis B.*" These have since been rediscovered, redescribed, and renamed *Chilomastix* and *Trichomonas*. Other forms belonging to the same group of organisms have also been found and studied by Davaine's followers down to the present day.

A much larger animal was found in human stools by the Swedish physician Malmsten in 1856. His organism differed so strikingly from those already mentioned that it clearly belonged to a different group. It is now called *Balantidium* and has been studied and redescribed by many later workers. About the year 1860 another Swede, Kjellberg,* discovered yet another different kind of organism, this time living actually in the tissue of the human bowel. This was the first of the animals now called Coccidia to be described in the human gut; and its discovery has been followed by the finding of several similar forms which have received the attention of many subsequent investigators.

Finally, a fourth kind of "little creature" was discovered † in human stools by two Anglo-Indian medical officers, Lewis and Cunningham, in the years 1870 and 1871. Soon afterwards—in 1875—a similar discovery was made by Lösch in Russia. The organisms which these observers studied are known as amoebae, and belong to a different group of animals from any of those previously noticed. They have now been very thoroughly studied by later workers, and their numbers have been augmented accordingly.

The discoveries briefly related above are all landmarks in the subject with which the present work deals. They mark the beginning of our knowledge of four different groups of microscopic animals which inhabit the human bowel: and the following up of these several discoveries has resulted in the accumulation of an immense mass of facts which now almost form a special science by themselves. It is now known that all these animals belong to one great group of the animal kingdom—the Protozoa—of which they form, however, an almost infinitely minute part. That they have attracted so much attention is due to the circum-

* The discovery was reported by Virchow in 1860. See Dobell (1919) for further details.

† This discovery is usually incorrectly attributed to Lambl (1860). Cf. Dobell (1919 *a*, pp. 8-9, and 71 *et seq.*) where additional details will be found.

stance that some of them—like the organisms found by Malmsten and Lösch—are associated with human diseases : and although only a few can claim this unenviable distinction, it has inevitably invested the others also with a particular human interest. Some conception of the present magnitude of this branch of Protozoology can be formed from the scope and size of the present volume.

Having made the foregoing brief allusion to the history of our subject, by way of introduction, we shall attempt in the rest of this chapter to define, very briefly, the Protozoa : to survey, very rapidly, the forms which live in the human intestine : and to point out how these various forms live in this environment. Detailed descriptions will be given in later chapters.

THE PROTOZOA.—The Animal Kingdom is usually divided into two main groups, or sub-kingdoms—Protozoa and Metazoa. The latter group comprises all the animals whose bodies are built up of the morphological units called "cells," and may accordingly be defined as consisting of all the multicellular animals. The former group is usually defined, in contrast, as comprising the "unicellular" animals. For reasons discussed elsewhere (Dobell, 1911) the term "unicellular" appears objectionable and misleading; for it implies that the body of an individual protozoon is homologous with a single cell in the body of a metazoon, and not with a whole metazoal individual. If we regard the whole organism as an individual unit, then a whole protozoon is strictly comparable with a whole metazoon, and not with a part of it. But the body of a protozoon, though it often shows great complexity of structure, is not differentiated internally into cells—like the body of a metazoon. Consequently, it differs from the latter not in the number of its cellular constituents, but in lacking these altogether. We therefore define the Sub-kingdom of the PROTOZOA as the group which contains ALL NON-CELLULAR ANIMALS.

This is not the place to define "cell" and "animal": and we shall therefore entrust the comprehension of the foregoing definition to the common sense of the reader.

CLASSIFICATION OF THE PROTOZOA.—The Protozoa are classically subdivided into four main groups, which are generally called Classes, but which probably correspond more closely, in systematic status, to the groups called Phyla among the Metazoa. Various names have been proposed for these main groups, but we shall follow the usual conven-

tion and call them (1) Rhizopoda, (2) Mastigophora, (3) Sporozoa, (4) Ciliophora.

These four groups, or Phyla, of the Protozoa, can be roughly distinguished by means of the characters supplied by the external organs of locomotion of the animals placed in them. These characters have been used for classifying the Protozoa ever since 1773, when they were first used for this purpose by the Danish zoologist O. F. Müller. Modern protozoologists have found such simple characters inadequate, when used alone. Nevertheless, they will suffice for our present purpose, and will enable us to distinguish the four main groups as follows :—

(1) The Phylum RHIZOPODA comprises those Protozoa whose external organs of locomotion are typically *pseudopodia*—temporary prolongations or extensions of the protoplasm of the body, familiar to everyone as the means of movement in *Amoeba*.

(2) The Phylum MASTIGOPHORA consists of all those Protozoa which move, in their fully developed and typical condition, by means of whip-like filaments or *flagella*—familiar to all who have studied *Euglena*, or any other common flagellate.

(3) The Phylum SPOROZOA contains a number of exclusively parasitic forms, which in their motile stages—when present—move without the aid of any special external locomotory organs. The several common species of *Monocystis*, parasitic in earthworms, supply familiar examples—with their slow, worm-like motions, performed by the body as a whole.

(4) The Phylum CILIOPHORA contains all the Protozoa which move, in their typical active stages, by the agency of many little hair-like threads or *cilia*—exemplified in the familiar *Paramecium* and other common ciliates.

Each of these Phyla contains a vast array of species, variously collected into genera, families, orders, and higher groups. It will be unnecessary, however, to discuss their classification in detail here, and we shall limit ourselves to a consideration of the systematic position of those species alone with which the present work is concerned. It will suffice to note the general grouping of our forms, and their more obvious relations to one another.

The human intestine harbours protozoa belonging to all the four Phyla just enumerated. As these groups contain organisms as different

from one another and as distantly related as the members of different Phyla among the Metazoa, it will be clear that many of the intestinal protozoa of man have little but their habitat in common. Among themselves they show great diversities, which are expressed by placing them in different systematic groups. The Rhizopods in the human gut, for example, are closely related to the Rhizopods in the guts of other vertebrates and to those leading an independent existence in water: they are but remotely related to the Ciliates found in man, though they chance to share the same habitation. In other words, the organisms with which we have to deal form, as a whole, an "unnatural" group—in the systematist's sense—and are treated together merely because Nature has assembled them in a common domicile.

The Rhizopoda are represented in the human intestine by five species of amoebae belonging to four different genera—(1) *Entamoeba*, with two species *E. coli* and *E. histolytica;* (2) *Endolimax*, with one species *E. nana;* (3) *Iodamoeba*, and (4) *Dientamoeba*, each also with but a single species—*I. bütschlii* and *D. fragilis* respectively. All these belong to the Class called AMOEBAEA, which comprises all the naked rhizopods resembling the well known *Amoeba* and its allies.

Among the Mastigophora, we find five distinct species—each belonging to a different genus—and several other doubtful forms which require further investigation. All of these belong to the Class FLAGELLATA—a very large group containing many families and genera. The genera and species found in the human gut are: (1) *Trichomonas hominis*, with several varieties, (2) *Chilomastix mesnili*, (3) *Giardia intestinalis*, (4) *Embadomonas intestinalis*, (5) *Enteromonas hominis*. To these some still uncertain forms may ultimately have to be added.

The Sporozoa of the human bowel all belong to the group known as the COCCIDIA, and are represented by four species placed in two different genera: (1) *Eimeria*, with the species *E. wenyoni*, *E. oxyspora*, and *E. snijdersi*, and (2) *Isospora*, with the single species *I. hominis*.

The Ciliophora found in man all belong to the CILIATA, a very large Class containing numerous species. Those of man belong to the genus *Balantidium*, represented by the species *B. coli* and *B. minutum* (somewhat doubtful), and possibly by others also. A species of another genus —*Nyctotherus*—has also been described, but its existence appears still rather uncertain.

All the organisms just mentioned will have to be considered in detail

in the ensuing chapters : but it will be convenient here to notice certain general characters which all the members of our "unnatural" group have in common. These concern chiefly their lives and habits, their distribution, and their relations to man.

LIFE-HISTORIES.—So many wonderful life-histories have been described—and even proved to occur—in the Protozoa, that the mere mention of the name often leads the less instructed to expect some marvellous revelation. It will be well to state at the outset, therefore, that the intestinal protozoa of man all lead—so far as we know at present—comparatively simple lives which can be understood, in their main outlines, by anybody. Most of them develop in a straightforward manner, and their development can be described without the use of numerous technical terms. Many of the exciting doings which have been attributed to these animals are now known to rest upon mal-observation, misinterpretation, and unscientific use of the imagination ; and no excuse will be needed, therefore, for ignoring these mistakes at this point, and omitting to use some of the superfluous terms which they have introduced into biological language.

From the most general standpoint, the life of an intestinal protozoon consists typically of two main periods—a period of freedom or activity (often curiously called a "vegetative" stage) and a period of rest. It may be noted, in passing, that the first period can hardly be called one of "freedom" and "activity" in the case of the Coccidia ; for during the corresponding stages in these organisms, the individuals are intracellular and sedentary—only the young forms being free and motile. But it is characteristic of all the intestinal protozoa that during this first period of relative freedom and activity they feed, grow, and multiply actively —multiplication being effected always by a process of simple or multiple fission. This period is, moreover, invariably passed—in the case of the organisms under consideration—within the human bowel. On the other hand, the resting period is always passed outside the human body, within a special protective capsule or cyst.

The "free" forms, living and multiplying in the body of man, give rise to the condition called infection : while the resting or encysted forms, capable of external existence, serve to convey infection from one man to another. Infection with any intestinal protozoon is, in nature, always acquired through the mouth, by swallowing a living cyst containing the resting form of the particular organism. In ordinary

circumstances the free forms cannot live outside the body for more than a very short time, and they die if swallowed—in other words, they are non-infective.

The two periods or cycles of development alternate, more or less regularly, with one another. When a cyst is ingested, it passes intact through the stomach into the intestine. Here it hatches and liberates its contained organism (or organisms), which seeks its appropriate place in the bowel and there begins its development as an active or free form. After living and multiplying for some time in this form, its offspring secrete cysts round themselves, and then pass out of the intestine with the stools. The cycle of events is repeated if these cysts are fortunate enough to get swallowed again by a human being.

The above is a brief outline of the life of each of the intestinal protozoa of man. Each has its own peculiar structure and mode of life, and each its own characteristic encysted form, which can be recognized in the faeces by the trained microscopist. Individual details of structure, and complications in the mode of development, will receive attention later. It is only necessary here to take a very general view, and to emphasize the two main stages in the life-cycle—the "active" form and the cyst. When these are understood, the details are easily learned: but failure to understand these simple generalities has led, unfortunately, to many errors in the past, and for this reason it seems necessary to stress these elementary points. When they are clearly and generally comprehended it will become impossible for certain current but inaccurate expressions to survive. It will no longer be possible for a writer to describe a patient as "infected with cysts," or to speak of "cyst-carriers," or to ask for methods of medication which will "kill the cysts" in preference to the active forms—all which expressions, and others akin to them, are obvious absurdities.

It will be noted that the life-cycle as a whole requires but two environments—the human bowel, and some suitable resting place outside it. No secondary or intermediate host is necessary for the completion of the developmental cycle of any of the intestinal protozoa of man. In this connexion, however, it must be noted that other animals may assist in the dispersal of the cysts, and thus aid in spreading infections: and this leads us to consider the usual modes of dissemination of the intestinal protozoa of man in nature.

Dissemination.—The cysts of all the intestinal protozoa of man

are comparatively delicate structures, and their contents are incapable of withstanding desiccation. In damp faeces, however, or in water, the cysts can usually survive and remain infective for several weeks. It thus seems probable that, in nature, water plays an important part in their dissemination : and it may be assumed that the swallowing of water, or damp uncooked foodstuffs, accidentally contaminated with faecal matter containing cysts, is the usual means whereby infections spread from man to man. All unhygienic conditions which favour conveyance in this manner must, accordingly, be regarded as contributing to the dissemination of infections.

Food and drink may, of course, become contaminated with faeces in innumerable ways. It is impossible to discuss them all here, but we must mention one of them which is of special interest—namely, contamination by flies. It has been demonstrated by Wenyon and O'Connor (1916, 1917), Flu (1916), Buxton (1920), and others, that house-flies are able to spread the cysts of the common species of intestinal protozoa. Wenyon and O'Connor have shown that a fly, when it feeds upon human faeces containing cysts, does not digest them, but passes them alive and unchanged through its alimentary canal, and voids them again—still living—with its own faeces. The time taken in passing through the fly is, sometimes, astonishingly short—cysts taken in at the fly's mouth being redeposited within as little as 5 to 30 minutes. A large number of flies, after feeding upon a stool containing numerous cysts, might therefore disseminate them over a comparatively wide area in a short space of time. Each speck of such fly faeces, if swallowed with food or drink before it has time to undergo complete desiccation, is capable of infecting a human being. It is thus clear that the part played by flies in the spread of infections is not negligible, and may be of prime importance : and it is also evident that the destruction of flies, as a prophylactic measure against the spread of infections, merits serious attention.

It has recently been urged by Roubaud (1918) that the fly may, in reality, do more good than harm in this respect. He argues that, since the minute quantity of faecal matter deposited by a fly readily dries up, and any protozoal cysts which it may contain are thus killed, the fly may, in reality, contribute to the destruction rather than to the dispersal of cysts in nature. Infective faeces, when devoured by flies, is reduced to a fine state of division ; and the prompt desiccation which results renders the contained cysts non-infective in a very short time.

This consideration is, no doubt, of importance when we are considering what happens under hot and dry atmospheric conditions. When the air is humid, however, or when there are opportunities for the flies' faeces to be deposited in or on damp comestibles intended for human consumption, it is clear that we cannot—without further evidence—regard the activities of the fly as beneficial, or even as harmless. Recently Woodcock (1918) has attempted to show that the part played by flies—in the dissemination of amoebic cysts—is comparatively unimportant. He considers the humidity of the atmosphere to be the factor of primary importance determining the survival and dispersal of cysts. It is evident, however, that dampness of the air is the very factor which would prevent the faeces of flies from drying too rapidly; and consequently, even if it were proved that humidity is of great importance, it would in no way invalidate the conclusion that flies play a most important part in the dissemination of the intestinal protozoa of man. Both factors are, doubtless, intimately connected, and deserve careful consideration.

It may be noted here that Stiles (1913) had earlier suggested that the prevalence of intestinal protozoa in a community might be used as a criterion of the extent to which their food and drink are exposed to contamination with human faeces—as a measure of the effectiveness of the sanitary arrangements within the community: and Stiles and Keister (1913) have already attempted to utilize this criterion in the special case of the carriage of *Giardia* cysts by house-flies.

Geographical Distribution.—It is now certain that most of the intestinal protozoa of man are cosmopolitan in their distribution. They are not restricted, as is often assumed, to the tropics, but are known to occur in human beings in all parts of the world where search has been made for them. In all probability the gaps in our present knowledge, in this respect, make the distribution of some forms wrongly appear discontinuous; though it is possible, or perhaps even probable, that future work will show that some species—for example, the Coccidia—are limited to certain geographical areas. On the other hand, although all races of man have not yet been examined with this object in view, it is reasonably certain that most races of man harbour *Entamoeba coli* and *E. histolytica*—and probably the other intestinal amoebae—and all the common species of flagellates. These are known to occur in such widely separated places that it can hardly be doubted that their real

distribution is world-wide. It is also reasonable to conclude that all the common intestinal protozoa of man have lived in man for ages. They are not recent intruders but age-long companions of the human species.

There is nothing very novel in this wide geographical distribution, though it has but recently become evident: for the cosmopolitan occurrence of the Protozoa generally has long been a commonplace observation to zoologists.

One of the most interesting of the facts which have emerged from the recent activity in the study of the intestinal protozoa of man, is the demonstration that all the commoner forms occur, apparently indigenously, in the British Isles. Even *Entamoeba histolytica*—previously assumed to be more or less restricted to the tropics—has been shown to occur in no inconsiderable proportion of the inhabitants of these Islands. We owe the establishment of this fact chiefly to the work of Matthews and Malins Smith,* but their observations have been confirmed and extended by others. One of us has recently reviewed and summarized all the investigations undertaken to elucidate this problem, so that it will be unnecessary to deal with it in detail here.† It will suffice to note that most of the commoner species of known human intestinal protozoa occur at the present day in Britain—the most noteworthy forms which have not yet been recorded being *Balantidium* and the Coccidia.

It is clear that Britain cannot occupy an isolated position in this respect, and that further investigations will show that all the intestinal protozoa of man are much more widely distributed than was generally supposed until quite recently. Hitherto the greatest attention has been paid to the distribution of *E. histolytica*, but there are already sufficient records available to show that most of the other intestinal protozoa are at least as widely dispersed.‡ But we cannot discuss this subject in

* See especially Yorke, Carter, Mackinnon, Matthews, and Smith (1917), Matthews and Smith (1919, 1919*a*), Dobell (1921).

† See Dobell (1921).

‡ Among more recent contributions to this subject the reader may be referred to the following: Galliard and Brumpt (1912), Paviot and Garin (1913), Landouzy and Debré (1914), Bloch (1916)—French cases; Kuenen (1918)—Dutch cases; Fischer (1920)—German cases; Yakimoff (1917)—Russian cases; Kofoid, Kornhauser, and Plate (1919), Cort and McDonald (1919)—United States cases. There are also numerous other works dealing with the occurrence of intestinal protozoa in the inhabitants of temperate climates, but it would lead us too far to discuss—or even to attempt to cite—all of them. See also the papers on French cases of Balantidiosis cited on p. 119 *infra*.

detail here. We must pass on to the consideration of another important and equally large subject.

Incidence of Infection.—The recent Great War has fostered an immense amount of research upon the intestinal protozoa of man, and has led to the publication of a very large volume of records from all the chief theatres of military operations. It is impossible to attempt to summarize this work here, where we shall merely note what seem to be the most important general conclusions to be drawn from it.

The recorded findings as a whole—after due allowance has been made for the very considerable but inevitable proportion of errors contained in them—tend to show that intestinal protozoa are far commoner in man, in all parts of the world, than had previously been supposed. But at the same time they have revealed that these organisms are of less importance, from a medical standpoint, than was formerly believed. It has now become clear that the majority of the intestinal protozoa occur comparatively frequently in human beings everywhere; but that very few species are responsible for the causing of human diseases, and that none give rise to epidemics of such diseases. In the War, the amount of disease due to intestinal protozoa was, in all probability, when the number of individuals involved is taken into account, almost negligible.

Nevertheless, the hundreds of thousands of cases of intestinal disease which occurred in the course of the War afforded great opportunities for studying intestinal organisms of all sorts; and it is partly because so much importance was at first attached to the intestinal protozoa that the fact of their comparative unimportance has emerged. By far the most important intestinal protozoon, from the medical standpoint, is *Entamoeba histolytica*—the organism which "causes" the disease known as amoebic dysentery, and other pathological conditions. Special attention has therefore been directed to this parasite, and as a result we now have fuller information about it than about most of the other intestinal protozoa. It is certain that this amoeba occurs in a very considerable percentage of persons all the world over, and it is probable that at least 10 per cent. of the entire population of the globe is infected. The number may, indeed, be much higher. The majority of the other intestinal amoebae, and most of the flagellates, occur with at least equal frequency: and in the case of some of them—such as *Entamoeba coli* and *Giardia*—there is evidence to show that they are even more commonly present in mankind generally. These conclusions, by themselves,

indicate that intestinal protozoa must have relatively little pathological significance.

There is, however, some indication that all the intestinal protozoa of man occur with greater frequency in tropical aud subtropical countries than in temperate and cold ones. But it is still questionable whether this inequality of distribution has any direct relation to climate or temperature : it is probable that it depends primarily upon the more insanitary conditions and greater opportunities for the spread of infection which are present in hotter countries generally.

We can say no more on this subject here, and will make no attempt to summarize the published records dealing with the incidence of intestinal protozoa in the various races of man, and in the various armies engaged in the War.* Our space is circumscribed, and we have yet to consider some other topics of importance from a more general standpoint.

The Relation of the Intestinal Protozoa to Man.—It is most important that all who begin the study of the intestinal protozoa of man should rid themselves of any prejudices that they may have against so-called "parasites." This term is loosely used, in common speech, for any organisms that live inside other organisms ; and preconceived notions derived from this reproachful name have been responsible for much misunderstanding and confusion in discussing the protozoa of man. A few general remarks on this subject will therefore be made here.

Animals which live inside other animals are called collectively Entozoa, and those which harbour them are called their Hosts ; and a moment's reflexion will show that such an association of two organisms may be of divers kinds. It is clear that such an association may be beneficial to both host and entozoon, or harmful to both : or it may be beneficial or harmful to one member of the pair, and indifferent to the other. Let us consider each of these possibilities in turn.

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* Numerous references will be found in the *Tropical Diseases Bulletin*. The reader interested in this subject may be referred to the following recent works, which will also supply him with numerous further references to the immense literature dealing with the incidence of intestinal protozoal infections : Aubert (1917), Bahr and Young (1919), Baylis (1920), Bentham (1920), Boney, Crossman, and Boulenger (1918), Brumpt (1918), Chatton (1918*a*), Derrieu (1920), Dobell (1917), Dobell, Gettings, Jepps, and Stephens (1918), Dobell (1921), Flu (1918*a*), Lebœuf and Braun (1916), MacAdam and Keelan (1917), Mackinnon (1918), Matthews and Smith (1919*b*), O'Connor (1919), Ravaut (1917), Smith and Matthews (1917, 1917*a*), Wenyon (1916), Wenyon and O'Connor (1917). Hundreds of additional papers could easily be cited.

(1) When the association benefits both parties, the condition is one of Symbiosis—a not very frequent state in nature. An example is afforded by some of the flagellates living in termites ("white ants"). In return for the food and lodging which the termite gives to the flagellate, the latter helps the former to digest its own food. No such symbiotic arrangement appears to exist between man and any of the protozoa which he harbours in his gut.

(2) When the entozoon lives at the expense of its host, the phenomenon is known as Parasitism. The entozoon is a Parasite—in the biological sense—and is always more or less harmful. When the harm done becomes manifest, the host is said to suffer from a Disease, of which the parasite is colloquially—and therefore inaccurately—termed "the cause."

The intestinal protozoa of man furnish several instances of parasitism, and illustrate several different degrees of this condition. *Entamoeba histolytica*, for example, is a truly parasitic rhizopod, which lives upon its host's tissues. The man who harbours it never derives any benefit from its presence, but the amoeba itself is always vitally benefited. Sometimes the parasite, by its inroads into the tissues of the body, makes its host ill. He then suffers from a disease—dysentery—which is said to be "caused" by the parasite, and is called, in consequence, Amoebic Dysentery. A comparable condition is seen in the case of *Balantidium coli*. This ciliate also attacks the tissues, and "causes" the disease distinguished as Balantidial Dysentery.

In addition to the two organisms just mentioned there are all the Coccidia which live in the human bowel. All of these also are parasites—living at the expense of human tissue. But as a rule they do not "cause" any clearly recognizable disease, and their harmfulness is therefore less obvious. It should be remembered, moreover, that *E. histolytica* and *Balantidium coli* often appear to cause no obvious symptoms of disease, because their pathogenic capabilities are masked and therefore overlooked.

There is at present no clear evidence that any of the other intestinal protozoa are truly parasitic in man.

(3) There is a third condition which may be called Commensalism, in which the entozoic organism benefits from the association while its host is neither distinctly benefited nor harmed. This state is well illustrated by *Entamoeba coli* and other intestinal amoebae of man,

and by the common flagellates *Trichomonas* and *Chilomastix*. These animals feed chiefly upon the waste food-products and bacteria in the human colon. Lazarus-like they live upon the crumbs from the rich man's table. The food eaten by the host ultimately provides nourishment for the entozoic organism also, and in this sense the two feed in common. But although the association here is a vital necessity for the entozoon, it is of no moment to its host. It probably makes no difference to a man whether his faeces serve to support a *Trichomonas* inside his body or a brood of putrefactive bacteria outside of it.

It will be obvious that to stigmatize such inoffensive dependents as "parasites," and to regard them as dangerous producers of disease, is not warranted. By far the greater number of the so-called "parasitic protozoa" of the human bowel probably belong to this class of harmless commensals. It is, indeed, even possible that some of them are not merely inoffensive, but actually beneficial to their hosts : for in consuming waste products and bacteria in the large bowel they may play a useful part as scavengers. In this connexion we need not discuss the view, which is sometimes advanced, that they probably injure their host by the "toxins" which they excrete. It will suffice to note that the "toxins" of intestinal protozoa exist, at present, only in the imagination of those who regard with horror any organism which can be loosely termed a "parasite."

The organisms which we here call commensals are sometimes described as Saprozoic, because—like certain free-living (*i.e.*, not entozoic) forms—they feed upon decomposing organic matter. More often they are quaintly called "saprophytic"—a botanical term obviously inappropriate to animals. These terms have a significance too wide and inexact to denote the precise relation which we wish to imply here by the word "commensalism."

Between the true tissue-parasites and the commensals or scavengers like *E. coli*, there is a group of entozoa which may be called food-robbers.* These do not wait for the crumbs to fall from the rich man's table, but seize and claim a share of what is still—so to speak—on his plate. Among the intestinal protozoa of man, *Giardia* is a good example of this kind of hanger-on. This animal lives in the small intestine, and obtains its nourishment by absorbing a small

* We borrow this term—a very apt one—from Minchin (1912).

share of the food which has been partly digested, in this situation, by its host, for his own sustenance. It is questionable how far *Giardia* disturbs, in this manner, the bodily oeconomy of its host: but the amount of harm which it does is probably negligible, in ordinary circumstances, and it is clearly not easy to justify the contention that such an organism is a "dangerous parasite."

(4) The remaining possible types of entozoic habit may be dismissed in a few words. If both entozoon and host suffer ill consequences from their association, the combination cannot long survive as a normal and natural state. An individual instance of such a condition would be called a "disease"; and it would be pathological for the entozoon as well as for its host. As a normal relation between two species, it clearly could not become established. On the other hand, if the association positively benefited neither entozoon nor host, the relation would be casual, and not such a one as Nature would be likely to perpetuate.

Since the state of being infected with an entozoic protozoon is sometimes strikingly manifested by its results—for example, when the condition can be regarded as constituting a human disease—it has been found convenient to invent words to denote these states. Infection with amoebae is thus called AMOEBIASIS;* infection with Coccidia, COCCIDIOSIS; infection with *Balantidium*, BALANTIDIOSIS. We shall use these terms in discussing these conditions: but we would here make it clear that we do not use them necessarily to denote diseases—as is often done. Infection is not necessarily accompanied by clinical signs of disease; and to restrict the use of such terms to certain consequences of infection, rather than to the condition of infection generally, is not only inconvenient but also leads frequently to a misunderstanding of the true relations existing between an entozoon and its host, and their joint relations to the diseases which may result.

Infection with flagellates is sometimes called "Flagellosis," and some writers have gone so far as to distinguish infections with different genera of flagellates by distinctive terms. For example, infection with *Trichomonas* is sometimes called (*horribile dictu*) "Trichomonosis" or "Trichomoniasis"; whilst infection with *Giardia*—otherwise known as *Lamblia*—is called "Giardiasis" or "Lambliasis." Such terms are not

* This term—introduced by Musgrave and Clegg (1904)—is now in general use, and we therefore employ it. "Amoebosis" would be a more orthodox word, and philologically less objectionable.

only clumsy contraventions of the laws of language but also superfluities. At the present time it appears unnecessary to employ more than a single term for each type of infection—the types being determined by the zoological groups to which the particular infecting organisms belong. We shall therefore use a term such as Amoebiasis to denote infection with any kind of amoeba, and Coccidiosis for infection with any kind of coccidium. It would be absurd to subdivide Coccidiosis into the two conditions "Eimeriosis" and "Isosporosis" because man happens to be parasitized by coccidia belonging to the two genera *Eimeria* and *Isospora*.

Coprozoa.—The protozoa which live in the human intestine are usually seen, of course, in human faeces discharged from the body. Such material, however, forms a suitable medium for the growth and development of some of the free-living protozoa which usually live in decomposing organic infusions. These protozoa, which show a preference for faecal matter, but which do not live entozoically in the faeces while it is still in the intestine, are termed Coprozoic or Coprophilic. They cannot be regarded as parasitic or commensal, and their occurrence in human faeces is largely a matter of chance: for they occur at least equally often in the faeces of other animals, and in decomposing organic substances of many kinds. These coprozoic protozoa are of importance, however, because their occasional presence in stale human faeces has led to their confusion with the true intestinal forms.

Human faeces, after leaving the body, may contain coprozoic amoebae, flagellates, and possibly even ciliates: and every worker engaged in the study of the intestinal protozoa should make himself familiar with the commoner species. A brief account of some of these will be given in Chapter IX, and no further mention of them will therefore be needed at this point.

We shall now conclude this introductory chapter with a Table (p. 17), which gives a synopsis of the chief intestinal protozoa of man, and indicates at a glance their relations to one another in the zoological system as briefly noted in the preceding pages. The Table will also serve as a rough table of contents to the ensuing chapters.

Sub-Kingdom	Phylum	Class	Genus	Species
PROTOZOA	RHIZOPODA	AMOEBAEA	*Entamoeba*	*coli*
				histolytica
			Endolimax	*nana*
			Iodamoeba	*bütschlii*
			Dientamoeba	*fragilis*
	MASTIGOPHORA	FLAGELLATA	*Trichomonas*	*hominis*
			Chilomastix	*mesnili*
			Giardia	*intestinalis*
			Embadomonas	*intestinalis*
			Enteromonas	*hominis*
	SPOROZOA	COCCIDIA	*Eimeria*	*wenyoni*
				oxyspora
				snijdersi
			Isospora	*hominis*
	CILIOPHORA	CILIATA	*Balantidium*	*coli*
				minutum
			Nyctotherus	*faba*

Bibliographic Note.

The following note is inserted merely for the benefit of beginners, who are unfamiliar with the enormous mass of literature on the Protozoa.

The Intestinal Protozoa of Man are dealt with in most of the larger works on Tropical Medicine and on Protozoology or general Parasitology. In most of these, however, the descriptions are now out of date, and consequently incorrect or incomplete. Beginners are therefore likely to find the majority of such works puzzling and misleading rather than helpful. Much attention is also paid to the intestinal protozoa—both of man and of other animals—in the zoological text-books of Brumpt (1913), Minchin (1912), and Doflein (1916); and these works will be found useful for reference—especially Brumpt's admirable compendium. Even these are, however, already more or less out of date. Among the older works on the Protozoa, as a whole, by far the most authoritative and trustworthy is the monograph by Bütschli (1880-1889): but beginners, with no knowledge of more recent developments, are hardly likely to find this work very useful—though it is indispensable to every serious student of the group.

The intestinal protozoa of man have been considered collectively, as a separate group, by several previous workers, among whom may be mentioned Bensen (1908*a*), Wenyon (1915), and Brug (1918). Individual groups of these organisms have also been, from time to time, more or less completely monographed: the amoebae by Schuberg (1893), Craig (1911), Hartmann (1913), James (1914), Dobell (1919*a*), and others; the flagellates by Rodenwaldt (1912), Jollos (1913), etc.; the coccidia by Dobell (1919); and the ciliates by Jollos (1913*a*) and Prowazek (1914). Most of the recent works on intestinal protozoa have been reviewed in the *Tropical Diseases Bulletin* (London) and the *Bulletin de l'Institut Pasteur* (Paris)—the former published since 1912, the latter since 1902. For references to current literature these two periodicals will be found invaluable. Copious references to the literature of this subject will also be found in the *Zoological Record*, the *Zoologischer Jahresbericht*, and Stiles and Hassall's *Index-Catalogue of Medical and Veterinary Zoology*.

It may not be superfluous to point out, or to emphasize, in this place, that a sound knowledge of the Intestinal Protozoa of Man cannot be gained—even by a protozoologist expert in other branches of the science—by reading alone. To understand these organisms properly it is necessary to study them in the laboratory. Moreover, it is hardly possible to understand the forms living in man without some knowledge at least of those in other animals—and, in fact, without some grounding in the elements of the Science of Protozoology. The subject is a special one, and—like all special subjects—it cannot be properly grasped without some knowledge of general principles.

CHAPTER II.

THE INTESTINAL AMOEBAE OF MAN.

It has already been noted, in the preceding chapter, that the Rhizopod Protozoa are represented in the human bowel by five species of amoebae. These will be described in some detail in the present chapter.

Every elementary student of biology is familiar with an organism called "the Amoeba," or more boldly—if with less justification—"*Amoeba proteus*." Most of what he is taught about this animal is of more than questionable authenticity: but he probably learns, more or less correctly, that it—or something to which the name is applied—is a creature of apparently great simplicity of parts, having very few organs and leading a pleasingly simple life in ponds. The amoebae living in man are—at least superficially—similar organisms, but they are unfortunately not quite so simple as "the Amoeba" of the elementary text-book.

All the amoebae of man are of very small size. Their bodies are naked masses of protoplasm, of ever-changing shape during life. The organisms move and capture food by means of temporary extensions of their bodies (pseudopodia), and have few other noticeable organs—merely nuclei and cavities (vacuoles) containing ingested food. The organs known as contractile (or pulsating) vacuoles (or vesicles)—typically present in free-living forms—are invariably lacking. The protoplasm is differentiated, as in other animals, into nuclear and cytoplasmic parts; the nuclear apparatus differing in different genera and sometimes attaining some structural complexity, the cytoplasm being as a rule but little differentiated. The latter can always, however, be seen to consist of two parts—an inner and granular endoplasm forming the bulk of the animal, and a thin outer layer of clear

ectoplasm. These are the free forms of the organisms, which live inside the body of man, where they multiply by simple fission into two.

The encysted forms are derived from the free forms by the simple process of rounding off, eliminating all food, and secreting a delicate and transparent surrounding capsule or cyst wall. Inside the cyst other changes take place—especially nuclear multiplication, and the laying up of reserve food material—so that the fully-formed cysts often differ strikingly from the active amoebae. When fully formed, the cysts pass out of the body with the stools, and then remain in a resting condition for some time. If they are neither dried nor heated they can live outside the body for days or even weeks, but they undergo no further development unless they happen to be swallowed by a human being. They then pass intact through the stomach and into the small intestine, where they hatch and liberate their contained amoebae. These then pass on with the gut contents until they reach their proper place in the bowel—usually the large intestine—where they settle down and begin their entozoic life anew.

No conjugation or sexual process has yet been proved to occur in the life-cycle of any of the amoebae of man.

Owing to the apparent paucity of structures present in amoebae, the distinction of the various genera and species is often by no means easy. The most important structural characters are those supplied by the nuclear apparatus and the cysts. By means of these it is possible to distinguish the four genera and five species found in the human bowel with certainty. It will be most convenient, however, to describe each species first, and give a key to the genera and species —based on the nuclear and other characters—later, when the terms employed and the structures to which they are applied, are familiar to the reader. We shall therefore begin by describing each species separately, and will summarize the descriptions in tabular form afterwards (see p. 39).*

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* It should be noted here that throughout this chapter we follow the nomenclature and descriptions given elsewhere by one of us (Dobell, 1919*a*) : and in many cases we give, without discussion, the conclusions there reached. The reader desirous of further details is referred to that work. A synopsis of the genera and their chief synonyms will be found on p. 38 *infra.*

(1) *ENTAMOEBA HISTOLYTICA* Schaudinn, 1903.

Chief synonyms :

"Amoeba coli" Lösch, 1875.
"Amoeba dysenteriae" Councilman & Lafleur, 1891.
"Amoeba coli felis" Quincke & Roos, 1893.
Entamoeba dysenteriae (Counc. & Lafl.) Craig, 1905.
Entamoeba tetragena (Viereck) Hartmann, 1908.
Entamoeba minuta Elmassian, 1909.
Löschia (*Viereckia*) *tetragena* Chatton & Lalung-Bonnaire, 1912.
Entamoeba hartmanni Prowazek, 1912.
Entamoeba brasiliensis Aragão, 1912 (*pro parte*).
Löschia histolytica (Schaudinn) Mathis, 1913.
Entamoeba minutissima Brug, 1917.
Entamoeba tenuis Kuenen & Swellengrebel, 1917.

Entamoeba histolytica, the "Dysentery Amoeba," was discovered in Russia by Lösch (1875), in the stools of a patient suffering from dysentery. In the period of nearly half a century which has since elapsed, it has been studied and described by a large number of workers : and, as a result, it is now the most fully investigated and best known of the species living in man.

The ACTIVE AMOEBOID FORMS of this parasite (see Frontispiece, and Pl. II, fig. 1) usually measure 20 μ to 30 μ in diameter when rounded and at rest. Their endoplasm is colourless, finely granular, and uniform in appearance : their ectoplasm clear and well developed.

The single nucleus is a delicate vesicle, inconspicuous or invisible during life. When carefully fixed and stained, it is seen (Pl. II, fig. 1) to have the following structure. The nuclear membrane, which is very thin, and achromatic, is lined internally with a layer of fine chromatin granules—usually in contact with one another. They are usually of nearly equal size, so that the nucleus appears in optical section as a finely beaded ring. At the centre of the nucleus there is a small structure, the karyosome, which consists of two parts—an inner granule or tiny sphere of chromatin, surrounded by a clear achromatic zone. In the stained nucleus an achromatic network, free from chromatin granules, fills up the space between the karyosome and the peripheral layer of beads. The nucleus as a whole usually measures from 4 μ to 7 μ in diameter—according to the size of the organism. The chromatic

part of the karyosome has a diameter of about 0·5 μ or slightly more, but seldom attains 1 μ.

The mode of nutrition is peculiar in this species, being mainly by absorption and not by ingestion of solid food as in the more familiar free-living amoebae. Solid food is, however, at times ingested: but such food is also of a peculiar character. It consists entirely of red blood corpuscles and, more rarely, fragments of tissue-cells of the host. Red corpuscles are sometimes seen in large numbers, in various stages of digestion, in the endoplasm—more than a score being sometimes distinguishable: and these inclusions give the organism a very characteristic appearance (see Frontispiece). Bacteria and other particles in the host's faeces are probably never ingested by normal individuals.

The movements of this parasite are also very characteristic. A normal individual, just removed from its host, and examined in a suitable medium and under favourable conditions of temperature, displays astonishing activity. It flows, almost in a straight line, across the field of the microscope—in an extended form which suggests a slug moving at express speed. In this condition the anterior end consists of a single large pseudopodium, advancing so rapidly that no sharp line can be seen separating the ectoplasm from the endoplasm. The red corpuscles contained within such an organism flow about and roll round one another with every movement, as though the protoplasm were a mobile liquid. This rapid locomotion seldom persists for more than a very short time outside the body. The animal soon ceases to progress, and becomes more or less sessile. In this condition it usually continues to undergo pronounced changes of shape, accompanied by the emission of a few large, blunt, and blade-like pseudopodia. These pseudopodia are perfectly hyaline and highly refringent, and are composed entirely of ectoplasm—a fairly sharp line of demarcation being visible between their clear protoplasm and the granular endoplasm. (Cf. Pl. I.) Movements of this type may continue for hours, before the animal finally rounds up, ceases to move, and dies. No similar movement is performed under the microscope by any of the other intestinal amoebae of man.

Reproduction is effected by simple fission, which probably takes place as a rule in the tissues of the host. The process is illustrated in Pl. II, figs. 2—7. The nucleus first divides, by a peculiar method, forming first a spindle (figs. 2, 3), then a dumb-bell figure (figs. 4, 5),

and finally constricting into two (fig. 6). Fission of the cytoplasm then follows, resulting in the formation—after the daughter nuclei have undergone their reconstruction—of two daughter amoebae (fig. 7) exactly like their parent and equal to one another in size.*

ENCYSTATION, which takes place in the gut of the host, is accomplished in the following manner. The active forms pass from the tissues into the lumen of the gut, and there undergo one or more divisions, leading to a decrease in size—the size eventually attained by the daughter amoebae being proportional to the size of the cysts which they are about to form. At the same time they get rid of the red corpuscles or other food fragments which they may contain. As a result, peculiar small amoebae, with very clear protoplasm, are formed (Pl. II, figs. 8, 9). These are known as the PRECYSTIC FORMS of the parasite, and were described originally by Elmassian (1909) as a distinct species—*E. minuta*. They are, in consequence, still sometimes known as "*minuta*" forms—to distinguish them from the large active "*histolytica*" forms. The precystic amoeba is directly converted into a cyst by the simple process of rounding up into a small ball of protoplasm, and secreting a delicate and transparent capsule or cyst wall round itself.

The CYSTS of *E. histolytica* (Pl. II, figs. 10—16) are usually round or slightly ovoid structures measuring as a rule anything from about 7 μ to about 15 μ in diameter, though larger and smaller specimens may be found (*vide infra*). They were discovered by Quincke and Roos (1893); and rediscovered later by Huber (1903), Viereck (1907), Hartmann (1908), and Elmassian (1909), who mistook them for those of other species.† Schaudinn (1903), on the other hand, entirely overlooked them. Their development has now been studied by many workers—the first correct account having been given by Walker (1911).

When first formed, the cyst (Pl. II, fig. 10, and Pl. VIII, fig. A) contains a single nucleus, like that of the precystic amoeba. Its diameter measures about one third of that of the whole cyst. The wall of the cyst is thin, colourless, and uniform, and in a cyst of medium size has a thickness of about 0·5 μ. In addition to the nucleus, the cyst

* For a fuller account of the division of this species see Dobell (1919 *a*), p. 40 *et seq.* Division was probably first observed in *E. histolytica* by Harris (1894).

† Viereck (1907) regarded them as belonging to a variety of *E. coli*, and named this supposed variety *tetragena*. Hartmann (1908) called them *E. africana*, and Elmassian (1909) *E. minuta*.

typically contains two other structures—chromatoid bodies and glycogen vacuoles. The former—sometimes called "chromidial" (Schaudinn) or "crystalloid" bodies (Chatton)—are highly refringent rods or masses (Pl. VIII, fig. A^1) of a substance which stains deeply with chromatin stains (Pl. II, figs. 10—12, etc.). They are variable in shape and size (cf. figs.), and are sometimes absent. Occasionally they are present in the precystic amoebae, before they form their cyst walls; but as a rule they first make their appearance within the cyst.*

The glycogen vacuole appears in the living cyst (Pl. VIII, fig. A^1) as a faint clear area: but when the cyst is placed in iodine solution, it stains as a reddish brown patch, with an indistinct outline (Pl. VIII, fig. A^2). As judged by these appearances, the glycogen is not abundant in the cysts of this species. Sometimes more than one glycogen vacuole is present (cf. fig. B^3, Pl. VIII).

The development of the cyst is very simple, and consists merely in an increase in the number of nuclei. The originally single nucleus (Pl. II, fig. 10) divides into two (fig. 11), and each daughter nucleus again divides so that ultimately four nuclei are present (fig. 12). With increase in their number, the nuclei undergo a steady reduction in size—the four nuclei finally present having each a diameter of approximately one sixth of that of the whole cyst. The resting nuclei at all stages are like those in the full-grown amoebae; but they frequently show a slight concentration of the chromatin granules at one point on the periphery, so that the nuclear "ring"—in optical section—has a crescentic thickening on one side (cf. fig. C^3, Pl. VIII).

Mature cysts of this species are therefore 4-nucleate, and typically contain chromatoid bodies and a small amount of glycogen (figs. C^1, C^2, C^3, E^1, E^2, E^3, Pl. VIII). In this condition they leave the body. Outside they undergo no further development, but if kept in suitably damp faeces or water for some time, it can be seen that the chromatoid bodies and glycogen gradually disappear—both being used up, apparently, as reserve food material.

E. histolytica is a species which has a number of distinct RACES,

* Dehorne (1919) has recently described "crystalloids" in the adult amoebae present in liver-abscess pus. We have also observed these, but regard them as an abnormality—since the amoebae do not encyst in this situation. It may be noted that the figures of Dehorne depict degenerate amoebae (as shown by their nuclei), and that the "crystalloids" in them resemble crystals rather than chromatoids in many cases (? Charcot-Leyden or haematoidin crystals).

distinguishable by the size of their cysts. The diameter of the cysts formed by a given race is variable, but the average diameter constant : and races have now been shown to exist whose cysts measure from 6·8 μ to 15·0 μ in mean diameter. The commonest races are those with cysts averaging 7-9 μ or about 11·5 μ to 13·5 μ. Examples of such races are shown in Pl. II, figs. 10-16 (3 races), and are strikingly compared in Pl. III, fig. 28. (See description on Plate.) So far as is known at present, the races differing in the sizes of their cysts differ from one another in no other character—either morphological or physiological. Such races were first described by Wenyon and O'Connor (1917), and have been investigated in detail by Dobell and Jepps (1918).* The races with the smallest-sized cysts have been erroneously described and named as distinct species by several workers—*e.g.*, by Prowazek (1912*a*) who called them "*E. hartmanni*," Kuenen and Swellengrebel (1917) who called them "*E. tenuis*," and Brug (1917*a*) who called them "*E. minutissima*."

If the ripe cysts—belonging to any race—are swallowed by a human being, they probably pass intact through the stomach : but on reaching the small intestine they hatch, and liberate their contents. The details of this process require further study : but there is some evidence that each cyst liberates a single 4-nucleate amoeba in the small intestine, and that its division into four small uninucleate amoebae—which establish the new infection—takes place subsequently (Chatton, 1917*b*). The cyst walls are insoluble in gastric juice, but soluble in trypsin (Ujihara, 1914) ; and it is certain that the cysts never hatch in the colon, where they are formed, or outside the body. Whatever the early stages of development may be, it is clear that the young amoebae from the cysts must pass on rapidly into the large bowel, where they soon establish themselves in or on the mucous membrane. They then grow—probably directly—into the large active forms previously described.

This completes the life-history, as far as it is known, of *E. histolytica*. Conjugation and "autogamy," described by some workers—most recently by Yoshida (1920)—have not yet been proved to occur, and

* The existence of such races is denied by Mathis and Mercier (1917), and also, apparently, by Nöller (1921)—but owing, as it would appear, to inadequate knowledge of the facts. On the other hand, Smith (1918, 1919) admits that such races exist, but considers that there is clear evidence of the existence of only two.

the published descriptions of such phenomena are clearly based upon misunderstandings of various sorts. If any sexual process occurs, it is probably in the earliest stages of the life-cycle, which are still imperfectly known: but the occurrence of such processes is still purely hypothetical. "Spore formation," of a peculiar kind, was described by Schaudinn (1903), but it is now certain that his description was incorrect.

Degeneration is very frequently seen in both the amoebae and the cysts passed in human stools, and has been frequently misinterpreted—degenerate specimens being regarded as new species or normal stages in development. It is impossible to describe all the degenerative stages which may be encountered, but the following points may be noted here. Degeneration in the active amoebae is at first chiefly noticeable in the nuclear structure. The peripheral chromatin beads become clumped into a few irregular masses on the internal surface of the nuclear membrane: the karyosome disintegrates: chromatin granules become scattered through the clear zone between it and the periphery: and finally the whole nucleus may break up and disappear. Degeneration of the cytoplasm is usually marked by vacuolation and bacterial invasion. In the cysts, similar degeneration of the nuclei occurs, while the chromatoid bodies disintegrate, the glycogen becomes diffusely distributed through the protoplasm, and the latter becomes progressively vacuolated and bubbly in appearance. Finally the cyst presents the appearance of a very thin capsule containing only a few granules.

Abnormal development occurs sometimes, and results in the formation of more or less monstrous amoebae or cysts. The latter may contain abnormal numbers of nuclei (3 or 8), and show a variety of freakish shapes. Cysts containing more than four nuclei are, however, excessively rare: and for purposes of diagnosis their occurrence may be ignored.*

Further noteworthy points in the life-history and habits of this species will be considered in the next chapter. The other species occurring in man must now be briefly described; and in these descriptions reference will be made chiefly to those characters in which they differ from *E. histolytica*.

* Rodenhuis (1919*a*), among recent workers, has described the finding of a number of 8-nucleate cysts of this species. The proof that they really belong to *E. histolytica* is, however, by no means conclusive: and this applies to most of the similar records previously published.

(2) *ENTAMOEBA COLI* (Grassi) Casagrandi & Barbagallo, 1895.

Chief synonyms:

"Amoebae" Lewis, 1870; Cunningham, 1871.
Amoeba coli Grassi, 1879 (*nec* Lösch, 1875).
Amoeba coli mitis
Amoeba intestini vulgaris } Quincke & Roos, 1893.
Entamoeba hominis Casagrandi & Barbagallo, 1897.
Entamoeba williamsi Prowazek, 1911.
Entamoeba brasiliensis Aragão, 1912 (*pro parte*).
Löschia coli Chatton & Lalung-Bonnaire, 1912.

Entamoeba coli, the large harmless amoeba of the human colon, was probably first seen by Lewis (1870) and Cunningham (1871) in India. Since then it has been studied by very many workers. With the exception of its habits, and certain minor structural differences which serve to characterize it as a distinct species, this organism closely resembles *E. histolytica*—both morphologically and as regards its life-cycle.

The active adult AMOEBA (Pl. I, and Pl. II, fig. 17) is usually of about the same size as the corresponding forms of *E. histolytica*—*i.e.*, about 20 μ to 30 μ in diameter, when rounded. Its nucleus is also similar, but is distinguishable—in normal, well-stained specimens—by the following structural characters (cf. fig. 17, Pl. II). It is somewhat richer in chromatin, the peripheral layer being composed of slightly larger beads: the chromatic part of the karyosome is slightly larger, attaining a diameter of 1 μ in large individuals: the karyosome as a whole is correspondingly larger, and its position in the nucleus is almost always eccentric—not central, as in *E. histolytica*: furthermore, granules of chromatin are usually present in the comparatively clear zone between the karyosome and the peripheral layer of chromatin. (Compare the nuclei in figs. 1 and 17, Pl. II.)

In its mode of nutrition *E. coli* is radically different from *E. histolytica*, for it is a commensal organism feeding upon the micro-organisms and faecal fragments present in its host's colon. This difference is reflected in the constitution of its cytoplasm, which is bulky and granular and usually contains numerous food vacuoles charged with bacteria, yeasts, starch grains, vegetable débris, and other particles

derived from its host's faeces. (Cf. Pl. II, fig. 17, and Pl. I.) The vacuoles never contain red blood-corpuscles, however, or fragments of the host's tissues. In addition to the food vacuoles, others containing liquid are often seen. They have pointed ends, and are irregularly spindle-shaped, resembling gashes in the protoplasm. (Two are shown in the specimen figured on Pl. I.)

E. coli appears to be a voracious feeder, and it ingests not only food particles but even crystals, sand-grains, and other insoluble structures. It even ingests the cysts—and more rarely the active forms—of other protozoa present in its host's intestine. Individuals have been seen which have thus engulfed cysts of *E. histolytica* (Wenyon and O'Connor, 1917; O'Connor, 1919), *Giardia* (Grassi, 1888; O'Connor, 1919), *Isospora* (O'Connor, 1919), and free forms of *Giardia* and *Trichomonas* (Grassi, Casagrandi and Barbagallo, O'Connor, etc.). Ingested cysts or amoebae have also been noted recently by Cragg (1919), who appears to regard them—for reasons which are not clear—as playing a part in some kind of "conjugation."

The method of MULTIPLICATION in this species is probably, like that of *E. histolytica*, by simple fission into two. All the stages have not yet, however, been studied. A process of multiple fission, or "schizogony," has also been described (Schaudinn, 1903; Mathis and Mercier 1917*a*; and others); but the evidence is far from conclusive, and it appears probable that no such process really occurs in this species.

When freshly removed from its host, and examined under the most favourable conditions, *E. coli* sometimes displays considerable activity. As a rule, however, it appears extremely sluggish, and shows little locomotory movement—its motions consisting chiefly in changes of shape, without evident progression. At such times it has a characteristic appearance (see figure on Pl. I). No sharp line of demarcation separates the ectoplasm from the endoplasm; and the formation of large, clear, blade-like pseudopodia—so characteristic of *E. histolytica*—is never seen. (Compare figs. on Pl. I—Frontispiece.)

It should be noted here that degenerate, motionless, or dead specimens of *E. coli* are frequently indistinguishable from similarly abnormal forms of *E. histolytica*. The degenerate nuclei are often closely similar, and determination of the species from this character is therefore possible only when one is dealing with perfect specimens. Again, bacteria are so frequently present in dead and degenerate individuals of

E. histolytica that their presence in the cytoplasm is a character which must be cautiously considered when attempting to identify the species of a given specimen.

Cyst formation is preceded, as in *E. histolytica*, by the formation of PRECYSTIC AMOEBAE of smaller size than the adult forms (Pl. II, fig. 18). They are sluggish, free from all food inclusions, and contain relatively large nuclei. Their size is proportional to the size of the cysts which they are about to form. Precystic amoebae of this species are often almost or quite indistinguishable from those of *E. histolytica;* and degenerate specimens of either species can never, in practice, be identified with certainty, and are thus a constant source of difficulty in determination and of error in diagnosis.

The CYSTS of *E. coli*, which were first studied by Cunningham (1871), are similar to those of *E. histolytica;* but they usually are larger, have slightly thicker walls, and always contain, when mature, 8 nuclei. Development occurs in exactly the same way as in *E. histolytica*—namely, by a simple process of successive nuclear divisions, accompanied by decrease in the size of the nuclei as they increase in number. The cyst is thus at first uninucleate (Pl. II, fig. 19) and then successively 2-nucleate, 4-nucleate, and 8-nucleate (Pl. II, figs. 20-22). The resting nuclei, at all stages, are structurally similar to those in the full-grown amoebae. The 4-nucleate stage is probably of short duration, as it is the one least often seen in the stools; and when encountered, some of the nuclei are usually seen undergoing division (cf. figs. M^1, M^2, M^3, Pl. VIII).

Glycogen is almost always present in the cyst at certain stages, and is relatively abundant. It is formed in the uninucleate stage, and is very conspicuous in binucleate cysts. When these are placed in iodine solution, the glycogen appears as a solid mahogany-brown mass, with a sharply defined outline (see Pl. VIII, figs. K^2, L^2). At the 4-nucleate stage the glycogen is absent or scanty, and in typical 8-nucleate cysts it is rarely demonstrable (Pl. VIII, figs. M^2, N^2).

Chromatoid bodies are not conspicuous in *E. coli* cysts as a rule, but most cysts contain a few deeply-staining granules or irregular small bodies (Pl. II, fig. 22). At times, however, well-developed chromatoids are present. They are usually in the form of spicules, splinters, or filaments—often appearing as sheaves of spicules (Pl. II, fig. 25) or more rarely as coiled threads (Pl. II, fig. 24). The cysts with such inclusions

were described by Prowazek (1911) as those of a distinct species—"*E. williamsi*." Chromatoids were probably first seen in the cysts of this species by Grassi (1879*a*).

Occasionally cysts containing more than 8 nuclei are found in this species (Pl. II, fig. 26). Such supernucleate cysts are probably abnormal —some or all of the nuclei having undergone an extra division. Cysts with 16 nuclei (*i.e.*, double the typical number) are commonest: those with less (10, 12, etc.) or more (up to 20) being rarely seen. Some authors, but on very inadequate grounds, have regarded these supernucleate cysts as normal stages in the life-cycle.

E. coli, like *E. histolytica*, is a species divisible into numerous RACES distinguishable by the dimensions of their cysts. Cysts may be found of any diameter from 10 μ to 30 μ, or even more; but as a rule they measure from 15 μ to 20 μ. Matthews (1919) has shown that there are probably at least four distinct races, the average sizes of whose cysts are 15 μ, 16·5 μ, 18·7 μ, and 21·7 μ. In all these races there is, of course, considerable individual range of size around the mean. Fig. 23 (Pl. II) is a cyst belonging to a strain forming very small cysts, and may be compared with fig. 22 on the same Plate—from a race with cysts of larger and more usual size.

The remainder of the life-cycle of *E. coli* is probably like that of *E. histolytica*, but it has been insufficiently studied. It is certain, at all events, that the ripe cysts undergo no further development, after leaving the body, unless they happen to be swallowed by a human being: and it has been experimentally proved that infection is brought about by swallowing the cysts (Grassi, 1888; Calandruccio, 1890; Walker and Sellards, 1913). The cysts probably hatch in the small intestine, and liberate amoebae which establish themselves later in the colon.

Sexual phenomena have been described in this species, but have not been proved to occur. Schaudinn (1903) described a process of "autogamy" in the cyst—based upon a series of errors of observation. More recently Mathis and Mercier (1916*a*) have attempted to show that the cysts of this species are sexually dimorphic: but at present no good evidence has been adduced for "gamogony" or sexual differentiation of any sort.

(3) *ENDOLIMAX NANA* (Wenyon & O'Connor) Brug, 1918.

Chief synonyms :

"Small amoeba" Wenyon, 1912.

"Free-living amoebae" James, 1914 (*pro parte*).

Amoeba limax Wenyon, 1916 (*nec* Dujardin, 1841).

Entamoeba nana Wenyon & O'Connor, 1917.

Endolimax intestinalis Kuenen & Swellengrebel, 1917.

Vahlkampfia nana (Wenyon & O'Connor) Brug, 1917.

Endolimax nana, the common small amoeba of the human bowel, was probably seen by many of the earlier workers; but it is only in recent years that its individuality has been correctly recognized. Owing to its small size, it has often been mistaken for a young form of one of the larger species ; and because of its nuclear structure, it was frequently confused with the small species of coprozoic or free-living amoebae. It was first recognized as a distinct intestinal species by Wenyon and James, and was first named by Wenyon and O'Connor in 1917.

Like the preceding species, this organism appears to be quite harmless to its host. It feeds chiefly upon the small micro-organisms in the gut-contents, and does not attack the tissues.

E. nana usually measures, when rounded, about 8 μ in diameter—ranging from about 6 μ to 12 μ. The living organism (see Pl. I) is not as a rule very actively motile, when seen outside the body. It usually progresses slowly when first observed, and soon shows no movement save change of shape. Before long it rounds up and dies, and such rounded and more or less degenerate amoebae are the forms most commonly seen in the stools.

The most characteristic feature of this species is its nucleus, which has a structure very different from that seen in the genus *Entamoeba*. The nucleus is vesicular, with a delicate membrane, free from chromatin, and measures from about 1 μ to 3 μ in diameter in fixed and stained specimens. (See Pl. IV, figs. 29—32.) All the chromatin is contained in a relatively large and typically irregular karyosome, which shows great variation of form in different individuals (cf. figs.). The karyosome usually consists of one fairly large and eccentrically placed mass, connected by strands or processes with other smaller masses or granules. The arrangement of these parts appears to be very variable, and it is

therefore impossible to regard any particular picture as "normal" or "typical." The types shown in the figures are all common, but by no means exhibit all the peculiar forms of karyosome seen in this species. Apart from the karyosome and its connected parts, no other chromatin structures or granules are visible in the nucleus. It should be specially noted here that in degenerate nuclei the chromatin is usually clumped in a single large mass, placed eccentrically at one pole.

The cytoplasm displays few noteworthy features. There is the usual clear ectoplasm, bordering the finely granular endoplasm, in which numerous minute food vacuoles containing ingested bacteria are usually present. Between ectoplasm and endoplasm no sharp line of division is, as a rule, distinguishable. The pseudopodia are few, blunt, and thick. (See fig. on Pl. I.)

It is probable that *E. nana* reproduces, like other amoebae, by fission into two. No complete description of the process, however, has yet been given ; and dividing individuals are extremely rare in the stools.

This species forms very characteristic CYSTS. The precystic amoebae—unlike those of species of *Entamoeba*—are not noticeably smaller than the ordinary active forms, from which they differ only in containing no food inclusions. They form colourless, thin-walled cysts which are usually oval—not round (Pl. IV, figs. 35-37). At first the cyst is uninucleate (fig. 35) ; and it then becomes, by successive nuclear divisions, binucleate (fig. 36) and finally quadrinucleate (fig. 37). Multiplication of the nuclei is accompanied by reduction in their size, so that the nuclei in the fully-formed cyst are very minute. Apart from this, the nuclei appear to be structurally identical at all stages of development. The cysts usually measure 7-9 μ in length.

Glycogen is occasionally, but by no means always, present in the cysts of this species. When present, it usually appears as a single mass, staining deeply with iodine. (See Pl. VIII, fig. H^2.)

Chromatoid bodies are absent from *E. nana* cysts ; but a few tiny bright granules are always visible in living cysts (Pl. VIII, figs. G^1, H^1, I^1), and these sometimes appear deeply coloured in stained preparations. From their microchemical reactions they appear to consist of volutin. In addition to these minute granular inclusions, the cysts rarely contain granular or filamentar structures, which stain deeply with iron-haematoxylin. Their nature is still uncertain (Pl. IV, fig. 38).

Supernucleate cysts, containing as many as 8 nuclei, are sometimes

found in this species (Pl. IV, fig. 39). They are, however, rare. Their nuclei are small and display the structure characteristic of the species, but the cysts themselves are generally of abnormally large size.

It is probable that there are several races of *E. nana*, distinguishable—as in *E. coli* and *E. histolytica*—by the dimensions of their cysts: but up to the present this has not been conclusively demonstrated.

Nothing is yet known of the early stages of development of this species. Presumably the cysts, when swallowed, hatch in the small intestine, and liberate small amoebae which establish a new infection. It is possible that the amoebae live in the small bowel, but the exact distribution of the organism in the intestine is still uncertain.

E. nana is sometimes parasitized by a minute micro-organism belonging to the genus *Sphaerita* Dangeard. The infected amoebae are very conspicuous, owing to the presence of the spore-morulae of the parasites in their cytoplasm (Pl. IV, figs. 33, 34). The spores appear as minute bright spheres, in the fresh state, but are usually coloured deeply in stained preparations.

(4) *IODAMOEBA BÜTSCHLII* (Prowazek) Dobell, 1919.

Chief synonyms:

Entamoeba bütschlii Prowazek, 1912.

"Spherical bodies" Wenyon, 1915. [Cysts.]

"Iodine cysts" or "I. cysts" Wenyon, 1916. [Cysts.]

"Pseudolimax" Kuenen & Swellengrebel, 1917.

Endolimax williamsi Brug, 1919 (*nec* Prowazek, 1911).

Endamoeba nana Kofoid, Kornhauser, & Swezy, 1919 (*pro parte*).

"*Endolimax pileonucleatus* Brug" Rodenhuis, 1919.

This species,* which has only recently become known, differs considerably in its nuclear structure and in the form of its cysts from those previously described. Its cysts were described under the name of "Iodine cysts" or "I. cysts" by Wenyon (1916), and became somewhat widely known by this name before their true nature was ascertained.

The active AMOEBAE of *I. bütschlii* (see fig. on Pl. I) resemble, when

* The species has recently been discussed—especially as regards its nomenclature—at great length by Nöller (1921) and Brug (1921 *a*). Their views do not lead me to suppose that what I have elsewhere written on the subject (1919 *a*) is in any way incorrect. Some of their opinions, on the other hand, are obviously untenable—*e.g.*, Brug's opinion that the organism may be called *Endolimax* by anyone who so desires. (C. D.)

alive, small specimens of *E. coli*. They are usually about 9-13 μ in diameter when rounded, but may measure anything from about 5 μ to 20 μ. They are generally but slightly motile outside the body, their movements being similar to those of *E. coli*. As a rule they degenerate and die very quickly after leaving the intestine.

The nuclear structure serves to distinguish this species with certainty, but it can be seen in perfectly fresh and very carefully fixed and stained specimens only. The nucleus (Pl. IV, figs. 43, 44) is a small vesicle with a diameter of about 2-3·5 μ. Its membrane is distinct and easily stained, and is usually achromatic though occasionally showing a very few minute darkly staining granules imbedded in it. The chromatin of the nucleus is, however, almost entirely contained in a single large central karyosome (cf. fig. 43), which is usually spherical, and intensely and homogeneously stained, but occasionally shows a paler centre (fig. 43). Between the karyosome and the nuclear membrane there is a "clear zone," which is almost filled with a single layer of small granules (cf. fig. 43). These granules do not stain as intensely as the karyosome with chromatin stains. On the other hand, they are readily coloured by plasma stains—such as eosin. Very careful fixation and staining, and a high magnification with well-adjusted light, are necessary to demonstrate their presence.

The cytoplasm displays few peculiarities. It is finely granular and homogeneous in appearance, and usually contains numerous food vacuoles charged with the minute bacteria upon which this amoeba feeds.

I. bütschlii probably multiplies in the bowel by simple fission, but the process has not yet been properly described. Dividing specimens are extremely rare in the stools. A few stages have recently been figured by Rodenhuis (1919).

The CYSTS of this species are very characteristic, and formed in the following way. PRECYSTIC AMOEBAE are first formed, differing in appearance from the ordinary active forms (Pl. IV, fig. 45). They are entirely devoid of food inclusions, and very sluggish. They possess, when alive, clear glassy-white protoplasm and a relatively large nucleus. When stained, it can be seen that the increase in the size of the nucleus is due chiefly to the increase in the number of granules lying in the clear zone between the karyosome and the nuclear membrane. (Cf. figs. 43 and 45.)

The precystic amoebae become more or less rounded, and then secrete their cyst walls. When fully formed, these are relatively thick, but colourless, as in the other species already described. Cyst formation is accompanied by important changes in the protoplasm. In the early stages, a patch of glycogen makes its appearance in the cytoplasm—staining at first palely and diffusely with iodine, but later becoming large, dense, and sharply contoured, and staining a deep mahogany brown with this reagent. (See Pl. VIII, fig. F^2.) In addition to this glycogen mass, other inclusions also make their appearance in the cyst. These are a number of small and brightly refractile granules (Pl. VIII, fig. F^1), which give some of the staining reactions of volutin. The glycogen mass and the volutin granules are the two typical cystic inclusions in this species—no chromatoid bodies being formed. The glycogen is sometimes absent, while occasionally two or even three separate masses may be seen.

In the mature cyst there is only one nucleus, but it differs in structure from that seen in the active form and the precystic amoeba. The granules in the "clear zone" become massed at one pole, whilst the karyosome is no longer central but closely pressed against the nuclear membrane at the opposite pole. (See Pl. IV, figs. 46—48.) It is usually large, and stains deeply ; and the nucleus thus has the appearance of a signet ring—especially when the cyst is examined in iodine solution Pl. VIII, fig. F^2).

The precystic amoebae and cysts of this species are not smaller than the active forms. The cysts, when fully formed, usually measure about 9-12 μ in diameter; but they are often difficult to measure, as they are subject to great variation in shape. Instead of being spherical, they are frequently more or less lobed or irregular (Pl. IV, fig. 48).

Living cysts appear white and hyaline, except for the dull area occupied by the glycogen mass, and the bright beads of volutin (Pl. VIII, fig. F^1). In iodine, the glycogen mass is their most striking feature (Pl. VIII, fig. F^2), but the nucleus also becomes visible. When fixed and stained, by ordinary methods, the nucleus shows its finer structure, the volutin grains are more or less distinctly visible, and the glycogen—being soluble in water—has disappeared, and its place is represented by a large vacuole (Pl. VIII, fig. F^3, and Pl. IV, figs. 47, 48).

Binucleate cysts are rarely encountered in this species; and they are probably to be regarded—like the supernucleate cysts of the other

species—as abnormalities. It is possible that this species is also divisible into a number of races distinguishable by the sizes of their cysts, but this has not yet been demonstrated. The cysts are so commonly irregular in outline that it is by no means easy to determine their diameters with accuracy.

Outside the body the cysts undergo no further changes, save that the glycogen—as in the other species—gradually disappears. When the cysts are swallowed, they probably hatch in the small intestine and liberate their contents as a single uninucleate amoeba which establishes the new infection. But the process has never been studied.

The cysts of this species have frequently been mistaken for those of other organisms. Flu (1918), for example, regarded them as degenerate cysts of *E. histolytica*, whilst Brug (1919) has referred them to "*E. williamsi*"—a name proposed by Prowazek (1911) for an organism which was chiefly, in reality, *E. coli*. More recently, Kofoid, Kornhauser, and Swezy (1919) have regarded *I. bütschlii* as a large race of *E. nana*—a view which is undoubtedly untenable. *I. bütschlii* is certainly a well characterized species, belonging to a genus which must be regarded—owing to its nuclear structure and cysts—as quite distinct from that of any of the other intestinal amoebae of man.*

(5) *Dientamoeba fragilis* Jepps & Dobell, 1918.

This is the smallest, and apparently the least common, of the amoebae of the human intestine. It was probably discovered by Wenyon in 1909, but rediscovered and first described by Jepps and Dobell (1918).

The ACTIVE FORMS (Pl. IV, figs. 40, 41) measure, when rounded, from 3·5 μ to 12 μ in diameter; their usual size being about 8-9 μ. They are actively motile, and show a distinct demarcation between their ectoplasm and endoplasm. The latter is finely granular, and usually contains numerous food vacuoles filled with tiny bacteria, upon which the organism feeds. When moving actively the amoeba has a snail-like appearance, its clear leaf-like pseudopods being in

* In a recent note it is stated by Nöller (1921) that *I. bütschlii* occurs in the pig. No evidence is adduced, however, to prove that the similar amoebae found in pigs are identical with those in man. The proposal recently made by Rodenhuis (1919) to change the name of the organism to "*Endolimax pileonucleatus* Brug"—an impossible combination, in any case—is contrary to the Rules of Nomenclature, and needs no discussion.

advance and its endoplasm massed in a somewhat concentrated " body " posteriorly (see fig. on Pl. I).

The most characteristic feature of this amoeba is its nuclear system. Unlike the other amoebae, already described, it is typically a binucleate organism (Pl. IV, figs. 40, 41). Its two nuclei are identical in structure, and may be placed either close together or more or less widely separated in the endoplasm. Their size is proportional to that of the whole animal, and ranges from 0·8 μ in diameter in very small up to 2·3 μ in very large individuals. As a general rule, each nucleus measures about 2 μ in stained specimens. The nuclei are spherical and vesicular, with extremely delicate limiting membranes. The chromatin is in the form of granules, of variable size, massed together in the centre of the nucleus so as to form a fairly large karyosome (Pl. IV, figs. 40-42). Between the karyosome and the membrane there is a clear zone, free from granules but crossed at intervals by extremely fine radiating strands of linin. Tiny granules can sometimes be seen in the nuclear membrane at the points where the linin threads join it.

About 80 per cent. of the amoebae of this species are binucleate—as just described. The remainder—mostly small forms—are uninucleate (fig. 42). Their nuclei are exactly like those of the binucleate forms in structure.

The amoebae of this species sometimes display peculiar fissure-like vacuoles in their cytoplasm (cf. Pl. IV, fig. 40). Outside the body they degenerate very rapidly, and in doing so become filled with much larger vacuoles, which give them a peculiar bubbly appearance. The vacuoles ultimately coalesce, and the whole organism then becomes a mere ring of protoplasm—in optical section—surrounding a clear central space. Such degenerate specimens can easily be mistaken for *Blastocystis*.

It is probable that this species reproduces by fission into two, but the details of the process require further investigation. It seems probable—from the occurrence of uninucleate individuals—that each binucleate specimen, when fully grown, divides, by simple fission of the cytoplasm, into two uninucleate individuals : and these young uninucleate forms then, during their growth, undergo a nuclear division so as to become binucleate once more. This interpretation is supported by the fact that organisms containing a single dividing

nucleus have been seen, but no specimens in which both nuclei are dividing. If this supposition is correct, then *Dientamoeba* differs in this respect from the other known binucleate rhizopods.

In spite of very careful search, no cysts of this species have ever been discovered. The rest of its life-cycle is therefore still in doubt, and its manner of conveyance from host to host is unknown. The organism is so delicate, and perishes so rapidly outside the body, that direct infection with active forms appears to be excluded.

It is not certain whether this amoeba inhabits the large intestine or the small, or possibly both. At present, from the evidence available, it seems probable that it lives in the colon. It appears to be a quite harmless commensal, and is thus more interesting to the zoologist than to the medical practitioner.

Determination of the Genera and Species.

Now that the amoebae of the human bowel have been briefly described, and their characters duly noted, we may give a key for the determination of the five species and their respective genera. This key will also serve to summarize, to some extent, what has been said in the preceding part of the present chapter. The species have been referred to four different genera, and we therefore give first a short summary of the synonymy of these genera for the guidance of the student.

Genera and Synonyms.

Genus 1. Entamoeba Casagrandi & Barbagallo, 1895.

Syn :

Poneramoeba Lühe, 1908.

Löschia, *Viereckia* } Chatton & Lalung-Bonnaire, 1912.

Proctamoeba Alexeieff, 1912.

Amoeba (pro parte), *Endamoeba*, *Entameba*, *Endameba*, etc. auctorum variorum [non *Endamoeba* Leidy, 1879].

Genus 2. Endolimax Kuenen & Swellengrebel, 1917.

Syn :

Entamoeba (pro parte) Wenyon & O'Connor, 1917, et aliorum.

Vahlkampfia Craig, 1913, et aliorum.

Genus 3. Iodamoeba Dobell, 1919.

Syn:

Entamoeba (pro parte) Prowazek, 1912, et aliorum.

Endolimax (pro parte) Brug, 1919.

Genus 4. Dientamoeba Jepps & Dobell, 1918.

Key to Genera and Species.*

1. (*a*) One nucleus present in active amoeba ... ... 2.
   (*b*) Two nuclei present ... ... Genus *Dientamoeba* 6.
2. (*a*) Nucleus with small spherical karyosome and peripheral layer of fine chromatin beads ... ... Genus *Entamoeba* 3.
   (*b*) Nucleus with large irregular eccentric karyosome, and no peripheral chromatin granules ... ... Genus *Endolimax* 4.
   (*c*) Nucleus with large central spherical karyosome, surrounded by a layer of achromatic granules ... ... Genus *Iodamoeba* 5.
3. (*a*) Ripe cyst 4-nucleate; glycogen diffuse; large chromatoids generally present ... . . *E. histolytica.*
   (*b*) Ripe cyst 8-nucleate; glycogen dense, in early stages only; large chromatoids occasionally present, but often absent ... *E. coli.*
4. Ripe cyst 4-nucleate; glycogen rarely present; chromatoids absent ... ... *E. nana.*
5. Ripe cyst 1-nucleate; glycogen in a dense mass; no chromatoids ... ... *I. bütschlii.*
6. Nuclei with central granular karyosomes, and no peripheral chromatin. [Cysts unknown] ... ... *D. fragilis.*

* This key is not intended, of course, as a complete diagnosis or description of the species and genera. It is founded merely upon the most striking and easily recognizable differential characters of the amoebae concerned.

CHAPTER III.

AMOEBIASIS.

THE term "Amoebiasis" was invented by Musgrave and Clegg (1904)* to denote a condition of "infection with amebas." We use it here in its widest sense, and define it as *the state of being infected with amoebae.* In the present context, of course, we use the term with special application to man: and as man harbours at least five species of intestinal amoebae, it will be seen that it may cover a variety of conditions. In practice, however, it is convenient to restrict the meaning of the word, and to use it more especially to denote infection with *Entamoeba histolytica.*

The reason for this is obvious. *E. histolytica* is a facultatively pathogenic tissue-parasite, and the state of being infected with it may be a diseased condition : at all events, it may be, and often is, a condition which is clinically recognizable. Infection with the other amoebae, which are probably all quite harmless, is not recognizable by any symptoms : and consequently a special word to denote infection with these species is rarely required.

Accordingly, the present chapter will treat especially of Amoebiasis in the restricted sense—the infection of Man with *Entamoeba histolytica,* and the consequences which this state may entail. It is impossible to deal with this large subject in detail in a work of the present scope, and we shall therefore confine our attention to the salient points.

PATHOGENESIS and AETIOLOGY.—The relation of *E. histolytica* to its host, and to the diseases which it plays a part in producing, may now be regarded as, in the main, accurately determined. We owe this chiefly to the work of E. L. Walker (1911, 1913), carried out in the Philippine Islands.

As we have often noted already, *E. histolytica* is a tissue-parasite. It

* By these authors the word is spelled "Amebiasis"—a spelling which is not agreeable with English usage.

lives upon living tissue only, and apparently cannot nourish itself in any other way. In the large intestine of man,* which is its usual home, it lives at the expense of the tissues forming the wall of the gut. The amoebae apply themselves to the mucous membrane, and secrete a powerfully cytolytic ferment which destroys the cells. The cytolysed tissue is then absorbed by the amoebae, and forms their chief food supply. More rarely they ingest solid fragments of tissue and blood corpuscles. By this process the lining of the gut is more or less eroded or ulcerated—the ulceration frequently extending into the submucous tissue, or even more deeply. It is thus clear that infection with this parasite must always produce a more or less pathological condition of the colon of its host.

As a rule, the damage done to the gut wall of the host is compensated by regeneration on the part of the tissues. These are able to keep pace with the inroads of the amoebae, and a condition of equilibrium is thus established between host and parasite. Such a state of equilibrium must be regarded as the "typical" or "normal" condition in *E. histolytica* infections. Neither host nor parasite suffers any appreciable harm from the arrangement: neither the one nor the other can be regarded as being in a definitely "diseased" state.

When the parasites and their host do not live in harmony with one another—as happens in a certain proportion of cases—pathological conditions result. These affect both the host and the parasite. In the case of the host, they are manifested as diseases, which may be classified into two main groups: (1) *Primary* or intestinal disorders, resulting from irritation of the intestine—most frequently manifested as diarrhoea and intestinal irregularities of various kinds, but leading in severe cases to a typical form of dysentery (Amoebic Diarrhoea, Amoebic Dysentery); (2) *Secondary* disorders consequent upon the wandering of the parasites from their primary site of infection, in the gut wall, into other organs—especially the liver†—where they give rise to inflammatory and suppurative conditions (Amoebic Hepatitis; Hepatic, Pulmonary, or Cerebral‡ Abscesses, etc.). All these diseased conditions of man are harmful to the parasite also; for they disturb

* *E. histolytica* was first found in the tissues by Koch in 1883 (see Koch and Gaffky, 1887) and shortly afterwards by Kartulis.

† *E. histolytica* was first found in the pus of hepatic abscesses by Kartulis (1887).

‡ The presence of the parasite in abscesses of the brain was first demonstrated by Kartulis (1904).

its food supply, interrupt its normal life-history, and lead to a great wastage and mortality among the amoebae concerned. In amoebic dysentery, for example, the amoebae are cast out of the body in large numbers before they can encyst; and they consequently perish and are unable to propagate their species. Similarly, in the case of secondary infections of the organs, the parasites, though they may enjoy a brief spell of reproductive activity in their new breeding ground, are doomed to extinction. They are unable to encyst in any situation save the gut, and from this site alone can they escape to the exterior; but in the internal organs they are cut off from the outside world with no means of continuing their race. The various amoebic diseases are thus "diseases" for the parasites as much as for their hosts.*

It will be clear that *E. histolytica* is a parasite which is by no means always recognizably "pathogenic." It always destroys its host's tissue, but by no means always gives rise to any outward manifestations of disease.

It should be expressly noted here that a patient suffering from amoebic dysentery, and passing large numbers of active amoebae in his bloody stools, is not infective to others. Ingestion of such active forms cannot, in nature, give rise to a new infection. Outside the body of man the amoebae are unable to encyst: they always perish ultimately, and usually very rapidly. But the healthy, or apparently healthy person, displaying no symptoms of his infection, is capable of infecting his fellows. In his gut the amoebae complete their normal development; and in his stools their ripe cysts pass out—ready to infect any other individual unfortunate enough to swallow them.

It will be understood that all factors which are conducive to the formation, preservation, dispersal, ingestion, and development of the cysts must be regarded as aetiologically important: but our space will not permit us to do more than mention the subject of general aetiology of amoebiasis at this point. It will also be evident that, from their very nature, amoebic diseases can never occur in epidemic form.†

Pathology and Morbid Anatomy.—As already noted, the ripe

* The foregoing paragraph is taken, with slight modification, from Dobell (1919*a*) p. 37.

† The "epidemics of amoebic dysentery" which have frequently been recorded are now known to rest upon mistakes of various sorts.

cysts of *E. histolytica* pass, when swallowed, unopened and intact through the stomach and into the duodenum. Here they probably hatch, and liberate their contained amoebae. The amoebae then pass on with the contents of the bowel into the great gut, where they proceed to establish themselves.

1. *Primary or Intestinal Amoebiasis.*—The large bowel is almost invariably the site selected for infection. Very rarely is the small intestine affected, though cases have been recorded by Harris (1898) and Kuenen (1909). Any part of the large intestine may be attacked, but the commonest parts are the caecum, flexures, and rectum. The vermiform appendix is sometimes invaded: and in long-standing cases the whole of the large bowel may be involved, with the exception of a small area immediately above the anal sphincter.

Our knowledge of the earliest changes which take place on infection is based chiefly on the results of experiments upon artificially infected cats and dogs. In sections of the gut of such animals it can be seen that the amoebae congregate on the surface of the healthy mucous membrane, and gradually erode it. They apparently secrete a ferment which dissolves the epithelial cells, and then come to lie in the pools of cytolysed tissue so formed. They do not dislodge the cells mechanically, or burrow actively into the healthy tissues. Frequently they pass down the crypts of Lieberkühn and attack the cells in this situation. At these early stages, when the condition is one of superficial erosion rather than ulceration, the affected areas of the mucous membrane appear to the naked eye as minute hyperaemic patches, owing to the dilatation of the surrounding capillaries.

Later, the initial lesion may give place to a very characteristic form of ulceration. The amoebae continue to multiply, and, as they do so, pass more deeply into the tissues. They may be arrested for some time by the muscularis mucosae, but ultimately they break through into the submucous layer. Here they continue the destruction of the tissue, invading it in all directions and so undermining the mucous membrane. The typical amoebic ulcer is formed in this way. It is a crater-like cavity in the lining of the bowel, its edges being well defined and overhanging, and the cavity itself filled with necrotic tissue which often projects in blackish shreds or tufts into the lumen of the gut. The amoebae are always found most plentifully at the periphery and base of the ulcer, in contact with the healthy tissue

in which it is formed. Such ulcers have been aptly compared with button-holes in the mucous membrane.

The ulcers, when solitary, may measure anything from a fraction of a millimetre up to several centimetres in diameter. Very frequently adjacent ulcers coalesce, and so give rise to a confluent type of ulceration which may involve a very large area. The ulcer may increase not only laterally in the submucous tissue, but also in depth —the amoebae finally reaching the muscular layers of the gut wall and penetrating these as far as the peritoneum. Perforation of the latter may ultimately occur, giving rise to peritonitis. The ulcers are usually surrounded by a halo of hyperaemia, but the mucous membrane between them usually appears healthy. Their edges are frequently swollen and embossed.

Histologically the following changes are seen. First, histolysis of all the tissues in contact with the amoebae: then dilatation of the surrounding capillaries, quickly followed by stasis and thrombosis: then some round-celled infiltration of the adjacent tissues: finally, a more or less extensive necrosis. Owing to the destruction of the capillary vessels, blood corpuscles are usually plentiful in the necrosed tissue, and free endothelial cells may also often be seen. Polymorphonuclear leucocytes are not typically present in or around amoebic ulcers; and when present in large numbers they probably indicate a secondary bacterial infection. The necrosed tissue in the ulcer cavity is a gelatinous coagulum containing cells in all stages of disintegration, and usually irregular lumps and globules of a chromatin-staining substance which is probably derived from the nuclei of the destroyed cells.

The microscopic appearances of typical amoebic ulcers are depicted on Plate III, fig. 27, A, B, C. The drawings show a section through an early ulcer (A) in which the mucous membrane alone is involved; and a later ulcer (B) in which the amoebae have penetrated deeply into the submucous tissue. Fig. 27, C is a more highly magnified portion of the wall of the ulcer shown at B. It shows the amoebae in contact with the healthy tissue (below and to the left), and with the cytolysed tissues surrounding and behind them in the cavity of the ulcer (above and to the right). Red blood-corpuscles are present in some of the amoebae; and in this section (C) also, the deeply stained masses formed by chromatolysis of the nuclei of the necrosed tissue-cells, are conspicuous.

In such a typical and uncomplicated amoebic ulcer, the destruction of the tissue appears to be purely local and mechanical—by erosion and dissolution—and no obvious reaction on the part of the surrounding cells is visible.

In experimentally infected animals, amoebae are usually present in the ulcers and on the surface of the mucous membrane in vast numbers. In human material, however, they usually appear to be far less numerous. But there can be little doubt that this is a *post mortem* phenomenon—many of the amoebae having died and disintegrated before the tissue was fixed. Christoffersen (1917) found that when he took special precautions to preserve the amoebae *in situ*, they were as abundant in man as in animal infections—being so tightly packed, indeed, that they resembled "stones in a pavement."

Job and Hirtzmann (1916) have described an intracellular stage in the development of *E. histolytica* in early lesions; but no other workers have confirmed their observation, and we believe it to be erroneous.

As the amoebae pass through and destroy the tissues, these regenerate: and when a particular area has been forsaken by the amoebae, or when the latter have been removed by specific treatment, more or less complete healing takes place. Fibrous tissue is formed, and the mucous membrane and other layers are more or less replaced by this or by regenerated tissue. The scars of old ulcers have a characteristic parchment-like appearance, and are often of a greyish colour. Considerable thickening of the wall of the bowel, and, if the ulceration has been deep, the formation of peritoneal adhesions, are not uncommon sequelae.

The lymphatic glands which drain the infected areas are generally enlarged, and show inflammatory changes. In chronic cases they may become hard and fibrous.

2. *Secondary Amoebiasis.*—The destruction of the blood-vessels in the wall of the gut gives the amoebae an opportunity of entering the blood stream. When they gain access to the radicles of the portal vein they are sometimes carried by this vessel to the liver; and after colonizing this viscus they may pass from it into the general circulation, and so be borne to other organs—such as the lung or brain—in which they are capable of establishing themselves. It is in this way that secondary infections are generally brought about.

Amoebic Hepatitis and *Hepatic Abscess.* By far the commonest site

of secondary infection is the liver. When the amoebae reach this organ they attack its substance as they did the intestine: that is to say, they cytolyse and absorb the cells, and cause a more or less extensive necrosis. In early stages, this gives rise to hepatitis: but as the amoebae continue to destroy the tissue they finally cause the formation of an abscess in the liver. The necrosed tissue accumulates in the abscess cavity—having no outlet, like that formed in the gut wall—and forms the peculiar "pus" so characteristic of these lesions. This is not ordinary pus, but a viscous matter formed of necrosed tissue, containing cellular débris of all sorts, blood, some bile, and occasional crystals of haematoidin and cholesterin, with fat droplets. It is generally said to resemble anchovy sauce, but is far more stringy and viscid. It should be noted that, in typical uncomplicated cases, the "pus" from amoebic abscesses is bacteriologically sterile.

An amoebic abscess may be formed in any part of the liver, but its commonest site is the right lobe. It may be single, but often more than one is formed; and several small abscesses may fuse to form a single large one. At times these abscesses attain a very great size, becoming larger than a child's head and containing over a gallon of pus. They tend to enlarge upwards towards the diaphragm or forwards towards the abdominal wall. Unless evacuated by operation they may ultimately burst into the lung, or through the abdominal wall. Occasionally they rupture into the peritoneal cavity, or into the stomach, duodenum, colon, kidney, or inferior vena cava.

The amoebae are not distributed uniformly through the pus, but lie chiefly in contact with the healthy tissue at the periphery of the abscess, which enlarges in a centrifugal direction as a result of their inroads. Histological changes similar to those seen in the gut may be seen in the tissues adjacent to the amoebae. No pyogenic membrane is formed round the abscess; but old abscesses, after removal or destruction of the amoebae, may become encapsuled and fibrous, and finally calcified.

A small portion of the wall of an amoebic abscess of the liver is shown in fig. 27, D (Pl. III). Above, the amoebae are seen lying in and near the healthy liver tissue, with the necrosed tissue ("pus") in the abscess cavity occupying the lower part of the figure.

Pulmonary Amoebic Abscess. Amoebae may gain access to the lung either directly, from a liver abscess rupturing into it through the diaphragm, or indirectly by way of the circulation. An amoebic abscess

of the lung may then be formed, in a similar manner to one in the liver. The commonest site of such an abscess is the lower lobe of the right lung.

Lung abscesses often rupture into the air passages, and the pus is then coughed up. It is reddish and viscid, resembling that of a liver abscess ; but as a rule it is less copious, since pulmonary abscesses are rarely of large dimensions.

Cerebral Amoebic Abscess. Very rarely *E. histolytica* reaches the brain, and there gives rise to abscesses similar—*mutatis mutandis*—to those in the liver or lung. Such abscesses rarely attain a large size, and have hitherto always proved fatal. The cavity is filled with "pus"—necrosed brain tissue, etc.—and the amoebae are found, as in a liver abscess, imbedded in the wall. Only about 50 cases of amoebic abscess of the brain have been recorded.

Other lesions. Invasion of other organs or tissues by *E. histolytica* has been described. Cases of amoebic abscess of the spleen have been reported by Maxwell (1909) and Rogers (1913). Nasse (1892) reported the finding of amoebae in phagedaenic ulcers in the skin, and similar observations have been made more recently by Carini (1912, 1912*a*), Dagorn and Heymann (1912), Heymann and Ricou (1916), and others. If these observations are correct, it appears probable that *E. histolytica* usually infects the skin by way of the wound made for the purpose of draining a liver abscess. No cases of "natural" infection of the skin appear to be on record.

E. histolytica has been found occasionally in the urine. The first such case was described by Baelz (1883), who named the organisms "Amoeba urogenitalis." More recently, apparently authentic cases of "urinary amoebiasis" have been recorded by Fischer (1914*a*) and Walton (1915). It is still uncertain how the amoebae enter the urinogenital system, and what parts of it they are able to infect. Craig (1911, p. 233) states that, in a case which he studied, there was a minute fistula between the bladder and an amoebic ulcer in the colon. A number of other cases in which "amoebae" have been reported in the urine may be ignored here, as they appear to rest, for the most part, upon errors of observation and interpretation.*

Various complications which may follow amoebic infection—such as

* See Dobell (1919*a*), pp. 125–129.

strictures of the gut, peritonitis and appendicitis, bacterial infections of divers sorts, pleural and peritoneal adhesions, haemorrhages, etc.—can be merely mentioned here. Discussion of these conditions would take us too far afield.

Parasitology of the Lesions. It is most important to remember the relation of *E. histolytica* to the lesions which it forms, and the relation of the secondary infections to the life-cycle of the parasite. We therefore emphasize these points in terminating the present sketch of the Pathology of Amoebiasis.

The normal development of *E. histolytica* begins with the growth and multiplication of the active forms of the parasite : it ends with encystation and exit from the human body. To nourish themselves, grow, and divide, the active forms must destroy tissue ; and so long as the tissue is suitable, the amoebae will continue to feed upon it. Having fed for some time at the expense of the tissue of the gut, the active amoebae emerge from the ulcers which they have formed, and transform themselves into precystic amoebae and finally into cysts. This transformation usually takes place only in the lumen of the gut. When the active forms pass more deeply into the body, from their normal site in the gut wall, they may reach an organ—such as the liver—which will serve them as food. But in such a situation they are entirely cut off from the outside world. If they were to encyst in such places it would be of no avail, for the cysts would have no chance of emerging to infect a fresh host. They would merely perish *in situ*. What actually occurs is that the amoebae in the secondary sites of infection apparently make no attempt to encyst.* They continue to breed, in the active state, as though they had been transplanted into a rich culture medium. Their multiplication goes on until the whole brood is destroyed by the natural defensive mechanism of the host, by surgical interference, or otherwise.

If this is borne in mind, it is easy to understand how it is that different clinical conditions may present us with different forms of the parasite. The ordinary person infected with *E. histolytica* passes the cysts of the parasite in his stools. But he has the active forms of the amoeba in the tissues of his gut wall, and precystic amoebae in the

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* Mayer (1919) states that he has found cysts of *E. histolytica* in amoebic liver abscesses produced experimentally in two cats. Confirmation of this observation is required. If it is correct, it must be an unusual form of development in an abnormal host, and probably has no further significance.

contents of his intestine. If the amoebae irritate his gut sufficiently, he suffers from diarrhoea (Amoebic Diarrhoea). In his stools we then find, therefore, large numbers of precystic amoebae—often mixed with cysts in all stages of development. If the injury to the intestine is sufficiently severe, the patient suffers from Amoebic Dysentery. Blood and mucus escape from the ulcerated areas, carrying with them numerous amoebae from the damaged tissues. The amoebae now found in the stools are therefore the large active forms, often containing ingested red corpuscles. In typical cases of acute dysentery, precystic amoebae and cysts are absent from the stools. All infections of the organs resemble deep ulcers in the bowel. They contain large tissue-inhabiting amoebae only—never cysts or precystic forms : and the amoebae must be sought, in all such cases, in the living tissues forming the walls of the abscesses, and not in the necrotic tissue (" pus ") contained in them.

The foregoing points are well worthy of remembrance by all to whom it falls to examine stools or abscess matter for diagnostic purposes. To diagnose an amoebic disease with certainty it is not sufficient to prove that the patient is infected with *E. histolytica*. It must be proved also that the parasites are present, in the lesions concerned, *in their appropriate stage of development.*

SYMPTOMATOLOGY.—Clinical signs of infection with *E. histolytica* may be present or absent. When present, the infected individual may be extremely ill : when absent, he may be indistinguishable from normal healthy persons. All intermediate conditions may be encountered. We shall briefly note here the most important manifestations of amoebiasis, beginning with the subject of " carriers."

Carriers. The " normal " individual, when he becomes infected with *E. histolytica*, displays no definite symptoms of his infection. He lives in a state of equilibrium with his amoebae : for although they are continually consuming the lining of his colon, he is able to make good their depredations by continual regeneration of tissue. This condition is favourable to the amoebae ; for as long as their host can supply them with food, and as long as his bowels work in a normal manner, they are able to pass their lives in comfort. After multiplying in the wall of the bowel, they pass into its lumen ; and there, if their host does not empty his bowel too frequently, they have ample time to encyst, and so pass out—at the proper stage of development—with his stools.

A " healthy " person, infected in this way, is called a CARRIER of

the parasite. As he shows no outward and visible signs of his infection, he can only be distinguished, when alive, by the cysts of the amoebae which appear from time to time in his stools. We may define the carrier, accordingly, as the person who passes cysts of *E. histolytica* in his stools, but otherwise exhibits no outward signs of infection.

E. L. Walker (1911, 1913), whose work first gave us a precise conception of carriers, has subdivided them into two groups, which he calls *contact* and *convalescent* carriers. The former are persons who have never suffered any ill effects from their infections: the latter those who have displayed symptoms of disease, due to their infections, in the past, but who have since recovered and regained their health without losing their amoebae. Probably the vast majority of persons who become infected belong to the class of more or less healthy contact carriers.

It is important to remember that the term "carrier" is used in a somewhat peculiar sense in reference to amoebiasis. The term has a definite and special meaning, which differs from that with which it is often used by bacteriologists. It should also be remembered that if the host "carries" anything, it is obviously the amoebae in his gut wall. He is therefore correctly called a "carrier of *E. histolytica*," or an "amoebic carrier"; but to call him a "cyst-carrier"—as is all too often done—is obviously absurd.

There can be no doubt that the carrier of *E. histolytica*, though he display no symptoms, always has a more or less eroded or ulcerated gut. He frequently, indeed, has definite ulcers visible to the naked eye *post mortem*, as the observations of Musgrave (1910), Bartlett (1917), and others, have shown.

Carriers have a great practical importance, for they are the people who are responsible for spreading *E. histolytica*. They suffer no inconvenience themselves from their infections, and are therefore not suspected of harbouring the parasite: but they discharge the cysts—the only infective forms of the amoeba—with their faeces, and thus constitute a constant source of infection to others.

At times carriers may show slight symptoms referable to their infections, such as intestinal irregularities (diarrhoea, constipation, "indigestion," "debility," etc.): at other times they may become more definitely ill, and develop symptoms of dysentery or other amoebic diseases. This leads us to consider the symptoms of such conditions.

Amoebic Dysentery and Diarrhoea. The commonest intestinal symptom of amoebiasis is diarrhoea—Amoebic Diarrhoea, as we may term it. This ailment may develop at any time in a carrier of the parasite, or may manifest itself *ab initio*—as soon as the amoebae establish themselves in the body. It may be more or less severe and of variable duration. The stools are loose, and contain mucus but no blood—or very little, only recognizable by the microscope. Large numbers of precystic forms of *E. histolytica* are usually present in the stools, and frequently cysts also; and an occasional active form from the gut wall —containing ingested red corpuscles—may also be seen.

If the diarrhoea becomes more severe, it may develop into true dysentery—Amoebic Dysentery.* This disease may also arise suddenly, upon first acquiring infection, or may develop from a preceding carrier condition. It is characterized by bloody mucous stools, containing numerous active amoebae—many of them containing red corpuscles—but few or no precystic forms and no cysts. The dysentery is generally accompanied by tenesmus, and as many as thirty or forty stools *per diem* may be passed—or the patient may attempt to pass them, for in severe cases, though there is almost continuous straining, very little is evacuated. The patient has a tired, anxious, and drawn expression. His tongue is dry and furred, and his appetite impaired.

Physical examination shows a rigidity of the abdominal wall, and elicits a tenderness over the colon—often especially over the caecum. A cord-like thickening at the site of the lesion—due to spasm of the muscles of the gut wall—is often palpable. The temperature is generally normal, sometimes subnormal. At times, however, and especially at the beginning of a first attack, a slight rise of temperature may be noted. The pulse is at first normal, but if the disease continues, it becomes increased in frequency and diminished in tension. The blood count is also usually normal, though the contrary is often stated. (Cf. Low (1916), Fischer (1919), etc.)

The disease may be acute or chronic—an initial acute attack, if untreated, frequently subsiding into a most persistent and intractable form of dysentery. Fatal cases are not unknown, but are less frequent nowadays owing to better treatment. The patient becomes weaker and weaker, blackish sloughs appear in his stools—which often have a most

* This term was introduced by Councilman and Lafleur (1891).

offensive smell—and death results from exhaustion. It is often extraordinary how patients will, in spite of the pain and inconvenience, remain at their work and refuse to go to bed. In fatal cases they generally become exhausted gradually: but sometimes the patient collapses suddenly, and his condition may be choleraic in type. Haemorrhage may also occur, or death may result from peritonitis, due to perforation of the intestinal wall.

Amoebic dysentery is clinically classifiable into varieties called *fulminating, gangrenous, subacute*, etc.—designations which will be self-explanatory after what has already been said.

Secondary Infections. When the amoebae leave the gut wall and gain entrance to the liver or other organs, more or less severe symptoms of disease are always present.

Amoebic Hepatitis and *Amoebic Abscess of the Liver* may be regarded as early and late stages respectively of the same process. The symptoms of both are alike, but differ in intensity. They are chiefly enlargement and tenderness of the liver, with increased dulness on percussion; irregular fever—the temperature often showing a rise at nights; nocturnal sweats, and occasional rigors; and sometimes persistent cough, nausea or vomiting, and a trace of jaundice. A leucocytosis is generally present (up to about 20,000 total leucocytes per c.mm., of which 70 to 80 per cent. are polymorphs).

Dysentery may precede or accompany the formation of a hepatic abscess, but may also be absent. Not uncommonly, the formation of an abscess appears to arrest an attack of dysentery, or diminish its intensity.

It is a curious fact that amoebic abscess of the liver is much commoner in men than in women. It is also commoner in white people in the tropics than in resident natives, although the latter are probably more heavily infected, as a whole, with *E. histolytica*. The disease is uncommon in temperate climates, notwithstanding the fact that the parasite is of common occurrence.

Amoebic abscesses may attain a very large size, and unless treated surgically are frequently fatal. Sometimes, when left to themselves, they rupture spontaneously, and thus cause the death, or more rarely the recovery, of the patient. Even with proper surgical and therapeutic treatment, hepatic abscess is a dangerous disease.

Amoebic Abscesses in the *Lung* or *Brain* usually—but not invariably—

follow dysentery and hepatic abscess. The symptoms—*mutatis mutandis*—are similar to those of liver abscess and abscesses of the same organs resulting from other causes. Pain is usual at the site of the abscess—pain in the chest, in the case of a pulmonary abscess; headache, often severe, in cerebral abscess. Pyrexia, rigors, and night sweats are common symptoms; but with abscess of the brain the temperature may be normal. In the latter disease mental and nervous symptoms will, of course, also be present—according to the site of the abscess. The duration of the disease is usually short, and its termination death. A pulmonary abscess may, however, burst into the lung, and drain itself by this route—the pus being expectorated, and spontaneous recovery occurring.

It will be readily understood, from what has already been written, that a healthy carrier of *E. histolytica* is liable to develop symptoms of primary or secondary disease at any time. The amoebae are in his tissues, and in close proximity to numerous possible points of entry into the circulation. But we do not know at present what factors determine whether the amoebae stay in the gut or migrate into the internal organs by way of the blood-stream. An infected person may suffer from acute or chronic dysentery, but never show any signs of secondary infection. He may, on the other hand, get an abscess in his liver or brain without ever having suffered from dysentery or diarrhoea. A man may have a liver abscess almost as soon as the amoebae establish themselves in his intestine : but on the other hand, he may suffer from chronic amoebic dysentery for years, and suddenly develop a liver abscess at the end of the period. Convalescent carriers (see p. 50) are sometimes comparatively free from symptoms for long periods, and then suddenly relapse with dysentery, or develop secondary infections. At present there appears to be no law governing these conditions, and their irregularity and apparent inconsequence are extremely puzzling.

The available evidence shows that infections with *E. histolytica* are very persistent. When an infection is once acquired, it probably persists as a rule—unless eradicated by specific treatment—for the rest of life. (Cf. Wenyon and O'Connor (1917), Dobell and Stevenson (1918), etc.) There is no fully authenticated case of spontaneous cure on record. Consequently, all who once become infected with this parasite are liable to suffer from amoebic disease at some subsequent time. For the average case, however, the risk is probably small.

But few accurate data are available for the determination of the relation between the "carrying" period and the onset of symptoms, but the observations of Walker (see Walker and Sellards, 1913) have thrown some light on the matter. Walker experimentally infected 18 out of 20 men with *E. histolytica*. Infection—determined by the appearance of cysts in the stools—was established in from 1 to 44 days, the average period being 9 days. Four of the 18 infected men developed dysenteric symptoms subsequently, the times intervening between the infective feeding and the onset of symptoms being 20, 57, 87, and 95 days. The remaining men showed no symptoms during the period of observation—that is, 14 of the 18 remained contact carriers. It will be evident from these figures that it is impossible to define any "incubation period" in amoebic diseases.

In the foregoing experiments, it will be observed that 2 of the 20 men never became infected at all; and it should be added that some of the other 18 required more than one feeding with the infective material before they became infected. At present we know nothing about immunity to amoebic infection, but these observations suggest that there may be some kind of natural resistance to infection—a resistance which differs in different individuals.

All the evidence goes to show that whether an infected person suffers, or does not suffer, from his infection, depends rather upon his own susceptibility than upon the virulence of the parasite. The same strain of amoebae will produce dysentery in one host, and not in another: and the same strain in the same host may sometimes cause symptoms and sometimes none. Examples of this are furnished by Walker (1913), who experimentally infected a man by causing him to swallow cysts from the stools of a convalescent carrier. The second man became a contact carrier. From his cysts a third man was infected, who also became a contact carrier. But a fourth, infected from him, developed an attack of acute amoebic dysentery 20 days after ingesting the cysts. It seems evident, therefore, that the factors determining dysentery must be sought in the susceptibility of the host rather than in the pathogenicity of the parasite. At present there is no evidence to prove that different strains of parasites differ in "pathogenicity" or "virulence."

Reference has already been made several times to the wide geographical distribution of *E. histolytica*. It would seem to follow

that the amoebic diseases must be equally widespread: but it is a curious fact that the frequency of infection with the parasite, and the prevalence of amoebic dysentery and liver abscess, do not appear to coincide exactly or to run strictly parallel.

Amoebic dysentery and hepatic abscess are commoner in the tropics than elsewhere—as the frequent application of the epithet "tropical" to these diseases indicates. But they also occur in temperate countries—for example, in Britain. Yet with us they appear to be very rare, for scarcely a dozen authentic cases of indigenous amoebic dysentery have been recorded in the British Isles. On the other hand, there is now good evidence to show that a large proportion—probably between 7 and 10 per cent.—of the population of Britain is infected with *E. histolytica*:* and it is certain, therefore, that thousands of carriers of this parasite exist in our midst.

It is probable that the percentage of carriers in all tropical countries is higher than 10 per cent. How high it actually is, it is not yet possible to state for any particular country, since really accurate and extensive records are not available. It is clear, however, that the percentage of carriers in the tropics cannot be more than ten times as great as it is in Britain. On the other hand, amoebic diseases appear to be more than ten times as prevalent in some parts of the tropics. It is not easy, therefore, to reconcile these apparently conflicting facts.

Some would have us believe that residence in the tropics "conduces"—in some undefined way—to the development of amoebic disease in carriers of the parasite. Others speak of a particular state of the bowel, or the co-existence of certain intestinal bacteria, as the factors which determine the appearance of symptoms. Such "explanations," however, belong to the *ignotum per ignotius* category, and are not worth discussion until they can be formulated in more precise and scientific terms. At the moment it seems preferable, therefore, to suspend judgement upon this problem.†

Amoebiasis in Animals other than Man.—Many animals besides man harbour intestinal amoebae. It is not possible to discuss these organisms here; but it is necessary to say something about the experimental infection of animals with *E. histolytica*, since this subject is of present interest and of some practical importance.

* For a summary of the observations bearing upon this subject see Dobell (1921).

† See also on this subject Dobell (1921[b], p. 67, where a tentative explanation along biological lines is suggested.

E. histolytica has been successfully transmitted to dogs by Lösch (1875), Hlava (1887), Harris (1901), Dale and Dobell (1917), and others ; and an amoeba which appears to be identical has been found occurring spontaneously in these animals, in which it causes dysentery (Kartulis, 1891 ; Darling, 1915 ; Ware, 1916 ; Bauche and Motais, 1920). The cat appears to be more easily infected, but spontaneous infections of this host are not recorded. Among those who have studied *E. histolytica* in cats may be mentioned Kartulis (1891), Quincke and Roos (1893), Marchoux (1899), Wenyon (1912), Dale and Dobell (1917). Both cats and dogs appear to be most susceptible to infection when young. They may be infected by feeding them upon the cysts of the parasite, or by injecting active amoebae into the large intestine. When infection is established, it produces an acute and usually fatal dysenteric condition, similar to that seen in man. The amoebae appear to be incapable of encysting in these strange hosts : at all events, no authentic case of a dog or cat becoming a true carrier of the parasite is yet on record.

Amoebic abscess of the liver has also been produced experimentally in cats by Marchoux (1899),* Craig (1905), Huber (1909), Wenyon (1912), Dale and Dobell (1917), Mayer (1919), etc., and also in dogs (Harris, 1901). Kartulis has observed a spontaneous case of amoebic liver abscess in a dog.

Baetjer and Sellards (1914), and Chatton (1917*a*, 1918), have succeeded in infecting guinea-pigs with *E. histolytica ;* and Huber (1909) has succeeded in infecting rabbits. The lesions produced in these animals seem to be peculiar.

Lynch (1915*b*) claims to have infected the rat, though most other workers have been unsuccessful with this animal. Recently, however, Brug (1919*b*) states that he has found *E. histolytica* in wild rats (*Mus rattus*) in Java, and has also succeeded in infecting a rat experimentally by feeding it upon cysts from human faeces. If these observations are correct, as they seem to be, then it appears that the rat can become a true carrier of *E. histolytica*, for Brug found cysts of the amoeba in the faeces of his infected rats. Nobody has yet succeeded in infecting mice, or any other rodents.

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* In my book on the Amoebae of Man (1919*a*) I—like most other workers—unfortunately overlooked this important work of Marchoux. I am indebted to Prof. Mesnil for calling my attention to the omission. (C. D.)

Several species of monkeys, including anthropoid apes, harbour amoebae, which are not at present distinguishable with certainty from *E. histolytica* and *E. coli*. It is possible that they are the same species, but there is much uncertainty regarding their identity. They have been studied by Chatton (1912*a*), Mathis (1913*a*), Prowazek (1912*a*), Swellengrebel (1914), and others.* It should be noted that monkeys appear to suffer from spontaneous amoebic dysentery (Eichhorn and Gallagher (1916), and others), and also from liver abscess (Castellani, 1908).

None of the other amoebae of man can be experimentally transmitted to animals—so far as is known at present.† And for this reason, the rectal inoculation of kittens with the amoebae from human stools has sometimes been advocated as a means of confirming a diagnosis of *E. histolytica*. When successful, the result is conclusive : but when no infection follows, no conclusion can be drawn, since failure to infect cats with *E. histolytica* is a by no means uncommon consequence of such experiments.

The reader desirous of pursuing the subject of amoebiasis further may be referred to the following works, which deal with it from various aspects :

Councilman and Lafleur (1891)—especially for the early history and literature, and the classical account of the morbid anatomy. For later work, containing important corrections and additions, see especially Dopter (1905, 1907), Kuenen (1909), Christoffersen (1917), Dobell and Low (1921). A good recent account of amoebic abscess of the liver is given by Abriol (1917), and the works of Legrand (1912) and Armitage (1919) embody most of what is known about amoebic abscess of the brain. On carriers, and other general matters, see Walker and Sellards (1913) and Dobell (1919*a*). Clinical and other general information will be found in the books by Rogers (1913) and Phillips (1915), and in the larger treatises on tropical diseases—such as Manson's or Mense's well-known works.

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* The reader interested in them will find a fuller discussion of these interesting forms—with further references—in Dobell (1919*a*), p. 131.

† What appears to be a species of *Iodamoeba*—similar to *I. bütschlii*—has recently been described, under the name "*Endolimax kueneni*," from the intestine of a monkey (*Macacus cynomolgus*) by Brug (1921). Nöller (1921) avers that *I. bütschlii* itself occurs in pigs.

CHAPTER IV.

THE INTESTINAL FLAGELLATES OF MAN. "FLAGELLOSIS."

THE MASTIGOPHORA, or Flagellate Protozoa, are represented in the human bowel by at least five species—possibly by more. Three of these are common and well known. The rest are very small, and appear to be rare. Each species belongs to a different genus: but although these genera are now readily distinguishable, for the most part, and easily defined, there is still much confusion in their nomenclature. This is very largely due to the inadequacy of the earlier observations and descriptions, and the unfortunate bestowal of the name "*Cercomonas*" on all forms indiscriminately. It will help the beginner if he bears in mind that there is really no species belonging to this genus in the human bowel.*

We shall begin by describing the various species of flagellates found in the human gut, and will say a few words about their genera afterwards, as it will be easier to sort these out after the reader has become conversant with the organisms to which the generic designations are assigned.

(1) *GIARDIA INTESTINALIS* (Lambl) Alexeieff, 1914.

Chief synonyms:

"Dierkens" Leeuwenhoek, 1681.
Cercomonas intestinalis Lambl, 1859.
Dicercomonas (*Dimorphus*) *muris* Grassi, 1879 (1881).
Megastoma entericum Grassi, 1881.
Megastoma intestinale (Lambl) Blanchard, 1885.
Lamblia intestinalis (Lambl) Blanchard, 1888.
Giardia lamblia Stiles, 1915 [*in* Kofoid & Christiansen, 1915].
Giardia enterica (Grassi) Kofoid, 1920.

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* Species may, however, occur coprozoically in human faeces. They are considered on p. 178 *infra*.

Giardia intestinalis was discovered, in his own stools, by Leeuwenhoek, and described by him in a letter written to the Royal Society in 1681.* Later, it was rediscovered by Lambl (1859), who named it *Cercomonas intestinalis*. Grassi (1879-1888) devoted much attention to this organism, and gave it several different names. His final account of it, written in collaboration with Schewiakoff (1888), contains the first approximately correct and complete description.

As will be seen from the foregoing list of synonyms, the organism has received various names. Its nomenclature appears, however, to present no particular problem a present—the name which we here use being obviously the correct one,† though we may note that Kofoid (1920) has recently tried to justify the view that the correct name is *Giardia enterica*, and not *G. intestinalis*. This appears to rest upon an error. Kofoid states that the specific name *intestinalis*, proposed by Lambl (1859), is preoccupied, because Diesing (1850) had previously transferred Ehrenberg's *Bodo intestinalis* to the genus *Cercomonas*. But Ehrenberg's *"Bodo"* was really, in all probability, a *Hexamita* (Dujardin, 1841) :‡ consequently, Diesing's mistake does not render Lambl's name unavailable.

Giardia intestinalis (see Pl. V, figs. 58, 59) is a small flagellate with a very complicated structure. It is bilaterally symmetrical, all its organs being paired—right and left. Its shape may be roughly compared with that of a pear, from which a large slice has been cut off obliquely at the thicker end. The thicker end is the anterior, and the surface from which the slice has—according to our simile—been cut, is not in reality flat, but concave: moreover, it is not circular in outline but rather reniform—having an inpitting posteriorly. The whole of this area, which marks the ventral surface, thus forms a large cup-like depression at the anterior end of the body. It is supported round its edge by deeply stainable skeletal fibres, and acts as a sucker for the temporary attachment of the animal to the intestinal wall. This peculiar sucker-

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* See Dobell (1920), and compare Chap. I, p. 1 *supra*.

† The synonyms of the genus *Giardia* will be found on p. 86 *infra*. It may be noted here that in a recent paper Reuling and Rodenwaldt (1921) express a desire to retain the name *Lamblia* as a subgenus of *Giardia*.

‡ As pointed out some years ago by one of us (Dobell, 1909).

like organ is generally called the "sucking disc."* Its structure will be most readily comprehended from inspection of the figure on Pl. I and figs. 58 and 59 (Pl. V).

The hind end of the body tapers to a very fine tail, which, when the animal is alive and active, is usually recurved dorsally (Pl. I).

The length of the organism, from the rounded anterior extremity to the tip of the caudal process, is usually from 10 μ to 18 μ. The body as a whole is covered with a thin but tough pellicle, and is comparatively rigid. It shows no changes of shape save a slight bending of the tail, and some contraction and expansion of the sucker.

The internal structure is complex, and is in intimate relation with the flagellar apparatus. There are two nuclei, and four pairs of flagella, as well as certain fibres serving as a skeleton. The disposition and connexions of these parts are as follows (see Pl. V, fig. 58). The two nuclei are small oval vesicles, lying imbedded in the protoplasm at the anterior end of the body, dorsally to the sucker. In ventral view—as in fig. 58—they thus appear to lie within the sucker itself. Each nucleus has a deeply staining central karyosome (sometimes more than one), and a thin but well-defined nuclear membrane. Running lengthwise down the middle of the body there are two slender threads or rods—the axostyles—which are closely apposed for the greater part of their length. Anteriorly they terminate, before reaching the extremity of the body, in a pair of minute basal granules, or blepharoplasts: posteriorly they pass to the extreme tip of the tail, and there end in a similar pair of tiny blepharoplasts, in close contact. At the anterior pole of each nucleus there is also a minute granule, and from this a delicate thread passes to the blepharoplast at the anterior end of the axostyle on the same side. The nuclei are thus anchored, as it were, to the anterior ends of the axostyles.

A peculiar bar or block of a deeply stainable substance is often visible in the middle or posterior part of the organism. Sometimes it is comma-shaped or filamentar, sometimes double, and sometimes absent altogether. This is the "parabasal body" of some workers†—

* It is clearly improper to call the sucker a "cytostome," as has been done by Kofoid and Christiansen (1915) and some other recent writers.

† This structure was called the "darkly staining body" by Wenyon (1907) and "rätselhafter Körper" by Bensen (1908). Alexeieff, and Kofoid and his collaborators, suggested its homology with the parabasal bodies of other flagellates. It is somewhat difficult, however, to discover any grounds for regarding it as homologous with the

a structure of unknown function. It lies dorsally to the axostyles—which themselves lie near the ventral surface—and usually transversely to them. (Cf. Pl. V, fig. 59.)

The flagella are eight in number, arranged in four pairs. (See fig. 58.) (1) An anterior pair, arising from the blepharoplasts at the anterior ends of the axostyles, crossing over one another, and then passing round the antero-lateral margins of the sucker until they emerge at the sides as free flagella. (2) A middle pair, arising from—or very near to—the anterior blepharoplasts, then following the axostyles as far as the posterior margin of the sucker, where they diverge and pass backwards and outwards through the protoplasm until they emerge as free lateral flagella—springing from the sides of the body about midway between the posterior edge of the sucker and the tip of the tail. (3) A ventral pair, larger and more powerful than the others, arising out of the depression at the posterior edge of the sucker, and apparently rooted in thickenings of the axostyles themselves. These flagella often lie side by side for a part of their length, and lash in unison. (4) A caudal pair, long and very slender, arising from the minute blepharoplasts at the posterior tips of the axostyles.

The exact connexions and dispositions of these parts are extremely difficult to determine with precision, owing to their minute size and great complexity. Different individuals—differently stained, and lying in different positions—do not always present the same apparent structure ; and although we have examined a very large number of specimens (belonging to several species of the genus), we are still in some doubt regarding several details of the anatomy of this organism. The "axostyles," for example, sometimes appear rather as the walls of a tube, seen in optical section. They are usually connected at the anterior end by a somewhat indistinct structure interposed between the blepharoplasts (Pl. V, fig. 58.) Sometimes, also, there appears to be but a single axostyle, slightly split at the anterior end. The internal portions of the middle pair of flagella often appear to be quite continuous with their external or free portions : at other times there appear to be minute blepharoplasts—as in fig. 58—at the points where they emerge. The origins of the middle and the ventral pairs of flagella are also exces-

bodies to which Janicki (1911) originally gave this name. At present I regard the application of the name "parabasal body" to the structure present in *Giardia* as hardly justifiable—or at least premature. (C. D.)

sively difficult to determine. At times they appear to arise directly from the axostyles, at other times they seem to be given off from blepharoplasts lying upon the axostyles : and very often the caudal flagella appear to be simple prolongations of the axostyles themselves —no basal granules or other structures being visible to indicate a break in their continuity. We believe that many of the published pictures of *Giardia* are extremely schematic. They certainly do not all agree with one another in the points just noted,* and we find it difficult to reconcile the very plain diagrams which some authors have given, with the very puzzling appearances often presented by the organisms themselves. We therefore describe only the main structures with any confidence, and regard some of the details as still open to question.

Giardia intestinalis is an inhabitant of the small intestine—as Lambl first noted. The active flagellates live chiefly in the duodenum, but—judging by analogy with other species—they may be found scattered through the ileum also, as far as the ileo-caecal valve. In their natural surroundings they are probably actively motile, but they probably pass a considerable part of their existence attached by their suckers to the surface of the mucous membrane. In freshly passed stools, the organisms lash their flagella vigorously ; but they do not, as a rule, show rapid progressive movements—merely "skipping" up and down. They also show a tendency to attach themselves by their suckers to foreign bodies in the faeces, or to the microscopic slide or coverglass.

The cytoplasm of *Giardia* is remarkably free from inclusions of all sorts ; and as the animal possesses no mouth, it must be assumed that it obtains its nourishment by absorbing the partly digested food in which it swims in its host's intestine.

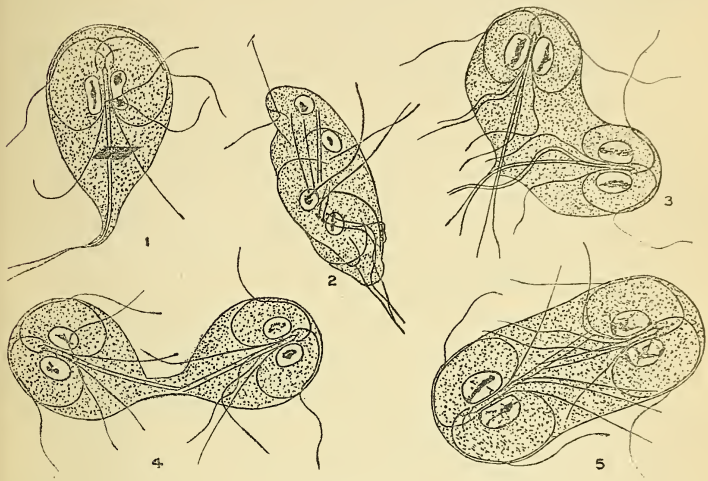
MULTIPLICATION is effected, as in other flagellates, by longitudinal binary fission.† It is very difficult to obtain individuals which are undergoing division, as they rarely appear in the stools. Moreover, the dividing forms are very difficult to interpret, owing to their great structural complexity.

Figures of various stages in the process have been published elsewhere by one of us in a joint work (Wenyon and O'Connor, 1917), and

*Cf., for example, the flagellar insertions figured (partly in other species) by Wenyon (1907), Bensen (1908), Kofoid and Christiansen (1915, 1915*a*), and Wenyon (1915).

†Noc (1909) describes multiple fission also, but his account and figures are far from convincing.

we reproduce them here (Text-fig. A). The finer details have not yet been completely elucidated, but the figures will give the reader some idea of the appearance of dividing organisms and of the complexity of the process. The nuclei appear to divide by mitosis, and the axostyles and other fibrillar structures apparently split to form those of the two daughter individuals.*



TEXT-FIG. A.

ENCYSTATION occurs in the lower parts of the gut. A single individual secretes a cyst wall around itself, and thus becomes completely encapsuled (Pl. V, fig. 60). The fully formed cysts have uniform and rather thick walls. They are oval, and measure 10-14 μ in length. At first the remains of the flagella and their fibrillar connexions are clearly visible inside the cysts, but later they disappear or break up. The two nuclei become detached from the axostyles, and soon undergo a mitotic division. As a result, there are then four minute nuclei at the anterior pole of the cyst (Pl. V, fig. 61). At the same time, the marginal fibres of the sucker become detached, and come to lie as very obvious crescentic bodies freely in the cytoplasm. They soon begin to split or fray

* Similar dividing forms of *G. muris* have been described and figured by Kofoid and Christiansen (1915*a*).

out, and the products of this multiplication or disintegration are then scattered through the cyst. The axostyles also split, and likewise the "parabasal" bodies, which grow in length and sometimes appear to break up. The final appearance of the contents of the cyst is thus very confused, and difficult to interpret. Some idea of the complex constituents of typical cysts can be obtained from figs. 60 and 61 (Pl. V), in which all the visible structures have been delineated as exactly as possible with the aid of the camera lucida.

The development observed within the cyst seems to be essentially a process of duplication of the organs preparatory to division. As a rule, however, complete division into two organisms does not occur within the cyst. The cysts present in the stools are usually binucleate (fig. 60), or quadrinucleate (fig. 61). Some authors have regarded the development within the cyst as involving a process of conjugation, but we believe such an interpretation to be unwarranted. There is no evidence at present of the existence of conjugation, or any other sexual process, in the life-cycle of this species.

The cysts of *G. intestinalis* were first noted by Grassi (1879*a*), who regarded them as being possibly those of coccidia. Later (1881*a*, 1888), he established their connexion with the flagellate forms. They are very characteristic structures, and cannot be easily confused with any other objects present in human faeces.

Several other points relating to the cysts may be briefly noted in conclusion. As will be seen from the figures, the protoplasm does not, as a rule, completely fill the cyst—a slight space being left at one or both ends. When placed in iodine solution, the protoplasm usually stains more or less brown (Pl. VIII, fig. P^2), probably indicating the presence of a small amount of diffuse glycogen. In every infection, however, if careful search be made, it will be found that certain cysts of this flagellate are stained a slatey blue with iodine. These cysts are usually very small (7-10 μ), and appear to contain degenerating organisms. It can be seen, moreover, that the blue colour is confined to the cyst wall—the contents being stained yellowish. This may, perhaps, indicate the presence of starch in the cyst wall. These small blue-staining cysts are very common, but we have been unable to ascertain their precise significance. We regard them as the results of some kind of degenerative process.

The encystation of *Giardia* probably takes place in the lower end of

the ileum, and possibly also in the large bowel. Like those of the intestinal amoebae, the cysts are unable to withstand desiccation, but will remain apparently unchanged in moist faeces for a week or two at least. They presumably hatch, when swallowed, in the small intestine and liberate two small flagellates from each—formed by completion of the division which begins in the cyst before it is discharged from the body. The earliest stages of development are, however, still unknown in this or any other species of the genus.*

(2) *TRICHOMONAS HOMINIS* Davaine, 1860, *emend.*

Chief synonyms :

Cercomonas [sp. 2] Davaine, 1854.
Cercomonas hominis (B) Davaine, 1860.
Cercomonas obliqua Moquin-Tandon, 1860.
"*Cercomonad* A" Cunningham, 1871.
Monocercomonas hominis Grassi, 1879.
Trichomonas intestinalis Leuckart, 1879 (*pro parte*).
Trichomonas hominis Grassi, 1888.
Cercomonas coli hominis May, 1891.
Tricomonas confusa Stiles, 1902.
Entamoeba undulans Castellani, 1905.
Hexamastix Ardin Delteili Derrieu & Raynaud, 1914.
Pentatrichomonas bengalensis Chatterjee, 1915.

The first recognizable notice of this organism is to be found in the work of Davaine (1854), who found it in the stools of a patient with typhoid fever. He referred it to the genus "*cercomonas*" (sic), and noted that it differed from a similar flagellate which he had found in the stools of cholera patients (=*Chilomastix*, in all probability†) in having its caudal filament inserted somewhat laterally, and in showing "an undulating movement" in its contours. Only a single anterior flagellum was made out. Later, Davaine (1860) published a figure of this species, but included it with the other (=*Chilomastix*) under the common name *Cercomonas hominis ;* though he distinguished them from one another as "varieties or species," called B and A respectively. The organism was

* The species most fully studied are those of rodents. Fairly full descriptions of some of these, with references to other works, will be found in the recent papers of Kofoid and Christiansen (1915, 1915*a*), and Boeck (1917, 1919). A species of this genus also occurs in the domestic cat, and another in tadpoles.

† See p. 71 *infra.*

subsequently referred to the genus *Trichomonas* Donné—by Leuckart and others—and its name therefore became *Trichomonas hominis* Davaine.* It has, however, been renamed by several later workers, as will be evident from the list of synonyms given above.

As a point of historic interest, it may be noted that this is the organism which Lambl (1860) found in human faeces and interpreted as an "amoeba"—a mistake which has won him the unmerited distinction of having discovered the intestinal amoebae of man.† The degenerating amoeboid forms of *Trichomonas* have been since described as "amoebae" by numerous other authors. It is also worthy of note that a very large proportion of the records of "*Trichomonas*" from man are based, in all probability, not upon this species but upon *Chilomastix mesnili*—a form which appears to be much commoner in human stools, and which has been regularly mistaken for *Trichomonas*.

Trichomonas hominis (Pl. I, and Pl. V, figs. 69-71) is a small and active flagellate. In shape it is usually oval; but its body is metabolic, and frequently becomes spherical, fusiform, or irregular. The length ranges from about 7 μ to 20 μ (living specimens), but most frequently lies between 10 μ and 15 μ—or less, when rounded. Posteriorly the body ends in a pointed caudal process.

Like the other species of the genus, this organism has a complicated structure. It is difficult to study accurately, on account of its small size: and it is an unfortunate peculiarity of this species that it is especially difficult to fix and stain. *T. hominis* usually shrinks considerably when fixed, and becomes more or less rounded; and its internal structures are difficult to demonstrate even by the best cytological methods. Well stained specimens show the following structures, which can mostly be made out—with perseverance—in the living organism also.

At the anterior end there is a single oval vesicular nucleus (Pl. V, figs. 70, 71), containing a small karyosome and a variable number of minute chromatin granules arranged on an indefinite linin network. It is bounded by a thin but definite nuclear membrane. At the anterior pole of the nucleus, and thus at the anterior tip of the body, there is a group of small blepharoplasts. It is extremely difficult to determine

* But see p. 71 *infra*, where Davaine's "*Cercomonas*" is further discussed. For the generic synonyms of *Trichomonas* see p. 86.

† Cf. Dobell (1919*a*) pp. 8-9, and 71 *et seq.*, and see also p. 2 *supra*.

their precise number, but there are at least three, and possibly more (fig. 71). They lie in contact with the nuclear membrane, and serve as points of insertion for a number of important organs—the flagella, the undulating membrane, and the axostyle.

The flagella are usually three or four in number (figs. 70, 71). They are very slender threads, of approximately equal size—their length being slightly greater than that of the body when rounded, or about equal to it when extended. They are all directed forwards, and are free for the whole of their length ; but they arise so close together that they often appear to be fused together towards their roots. In life they are lashed with great vigour. They appear to take their origin from at least two of the blepharoplasts.

The undulating membrane—one of the most characteristic features of the genus—is a longitudinally disposed frill or fin, which is supported by fibrous structures at its margin and its base. The margin is supported by a flagellum, which arises (see figs. 70, 71) from one of the blepharoplasts, and then passes backwards in an undulating line to the posterior end of the body, where it becomes free. The base of the membrane, at the point of attachment to the body, is supported by a stout and deeply stainable fibre, which may be called the basal fibre. This structure also arises from a blepharoplast anteriorly. It gradually tapers at the hind end, and terminates there in the protoplasm (figs. 70, 71). During life, the undulating membrane displays a continuous rippling motion, the waves passing rapidly along it from before backwards. It does not pass straight backwards from the anterior end, but is slightly wound round the body, and with the continuous rotation of the organism—when actively swimming—appears to lie first on one side, then on the other : and this, no doubt, explains why Davaine observed an undulating movement of the body "dans tout le contour." The membrane is widest in the middle, and narrows gradually towards its two ends.

The axostyle is a feebly stainable rod, which arises from one of the blepharoplasts, passes round the nucleus, and then lies centrally in the longitudinal axis of the body. It passes to the extreme hind end where it projects as a spike—forming, with the protoplasm investing its root, the caudal process. The axostyle is probably skeletal in function.*

* Kofoid and Swezy (1915), for other species, consider that the axostyle is itself actively motile. From very careful observations on a number of species I am satisfied that this view is incorrect. I believe that the view expressed above—which I put forward more than twelve years ago—is the correct one. (C. D.)

It is flexible, and appears to be passively bent with the movements of the body. It is difficult to demonstrate the exact relations of the axostyle to the nucleus and blepharoplasts in *T. hominis*. In other species, however, their connexions can be more easily made out.*

At the anterior end of the body there is a small slit, lying close against the nucleus. This is the mouth—seen as a crescentic mark in the figure on Pl. I, and in figs. 70 and 71, Pl. V. It lies towards one side of the body, which may be called ventral. The undulating membrane is on the opposite side, and may therefore be described as dorsal. It is difficult, however, to apply such terms as "dorsal," "ventral," or "lateral," to *Trichomonas*, as its body is slightly twisted, and not bilaterally symmetrical. Both mouth and membrane are somewhat obliquely or spirally disposed in relation to the long axis of the body.

The food of the organism, ingested through the mouth, lies in small food-vacuoles in the cytoplasm (fig. 71, etc.), where it undergoes digestion. It consists chiefly of small bacteria. Apart from food bodies, there are no inclusions in the cytoplasm. A permanent anus is lacking.

T. hominis is usually described as having three anterior flagella. Specimens with four or five are, however, also encountered. By some authors they are regarded as constituting distinct subgenera,† called respectively *Tetratrichomonas* (Parisi, 1910) and *Pentatrichomonas* (Mesnil, 1914; Chatterjee, 1915). The forms with five flagella have even been regarded as generically distinct from *Trichomonas*, and have also been named "*Hexamastix Ardin Delteili*," by Derrieu and Raynaud (1914).‡

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* In some of the larger species the axostyle appears to pass round the nucleus, in close contact with its dorsal surface. In others, I believe the axostyle completely encapsules the nucleus—as in *Lophomonas*—so that the nucleus really lies inside the expanded anterior end of the axostyle. It is possible that this is the case also in *T. hominis*, but I have not been able to determine the point with certainty. (C. D.)

† I regard these so-called subgenera rather as varieties. There seems, moreover, to be some misunderstanding regarding the use of these names. The type species of *Trichomonas* is *T. vaginalis* Donné, 1837. This, according to Kunstler (1883, 1884), Reuling (1921), and others who have studied it carefully, possesses 4 anterior flagella. Consequently, the 4-flagellate form being the type, no special name is required for it. "*Tetratrichomonas*" appears, therefore, to be superfluous. It is the 3-flagellate form which requires a distinctive name, and for this Kofoid (1920) has just proposed "*Tritrichomonas*." In my experience the 4-flagellate form (typical *Trichomonas*) is the commonest in human stools, but the 3-flagellate variety is also common. (C. D.)

‡ This generic name—as Mesnil (1915) has pointed out—is not available, as *Hexamastix* had previously been proposed for other flagellates by Alexeieff (1912*b*, 1914*a*). The same organism was named *Pentatrichomonas bengalensis* by Chatterjee (1915). The subgenus *Pentatrichomonas* was suggested almost simultaneously by Mesnil and Chatterjee. Their publications both appeared during the first few days of January, 1915, though Mesnil's is dated December, 1914. I do not know which of them has priority. (C. D.)

Degenerating "amoeboid" forms of *Trichomonas* are commonly seen in the stools. The organism loses its flagella and other organs, and then—without undergoing any appreciable locomotion—shows a series of curious changes of shape. A rapid undulation of a part of the surface is all that may be seen: but frequently a finger-like "pseudopodium" is thrust out at the anterior end, passes along the body towards the hinder extremity, and is finally drawn into the protoplasm in this region. The whole process may be repeated again and again for hours —or even days. Such degenerating individuals are very characteristic of *Trichomonas*, and do not really show much resemblance to amoebae. They have, however, been mistaken for such organisms by several workers. Lambl (1860) first described them as "amoebae," and Castellani (1905) named them "*Entamoeba undulans*." They were also noted—and correctly interpreted—by Cunningham (1871),* Roos (1893), and others.†

T. hominis multiplies in the bowel by longitudinal division; but stages in the process are excessively rare in the stools, and consequently no account of it can yet be given. The division of trichomonads is a complicated process, and conflicting accounts of the details, as observed in other species, have been published. It is impossible to discuss them here, and the reader is therefore referred to the original descriptions.‡

In spite of prolonged search by many workers, the cysts of *T. hominis* —if it form any—have not yet been discovered. The "cysts" attributed to this species by Prowazek (1904), and other workers in Germany, are, in reality, *Blastocystis* (see p. 141). Lynch's (1916) "cysts of *Trichomonas*" are really those of *Chilomastix*, and those observed by Boyd (1919) and others in "cultures" appear to be merely rounded and degenerating individuals. It is still uncertain how infection is conveyed from one human being to another. It should be added, however, that the cysts of some other species are known.§

* Cunningham called them "Cercomonad A," however, and was not aware that they belonged to the genus *Trichomonas*.

† Cf. pp. 2, 66, *supra*.

‡ See Prowazek (1904), Wenyon (1907), Dobell (1909), Mackinnon (1910, 1912), Kuczynski (1914, 1918), Kofoid and Swezy (1915), etc. In spite of the criticisms to which my work on *T. batrachorum* has been subjected by some of these authors, I believe that my observations were, in the main, correct. I hope to return to the subject on a future occasion. The work of Kofoid and Swezy, on related species, appears to me to be in some respects—*e.g.*, the behaviour of the axostyle—very unconvincing, and their figures and descriptions are by no means easy to reconcile. (C. D.)

§ The cysts of *T. batrachorum* were described by Dobell (1909), and those of *T. caviae* have recently been observed by Brug (1917) and others. The former are small and oval, the latter large and spherical.

Several authors claim to have succeeded in cultivating *T. hominis*, but some of the claims—such as that of Escomel (1913)—appear to be unjustified. It is probable that free-living flagellates, with which the cultures were contaminated, were mistaken for *Trichomonas*. Lynch (1915, 1915*a*) claims to have cultivated *T. hominis*, *T. vaginalis*, and the species found in the human mouth,* in acid broth. Boyd (1918, 1919), however, finds that *T. hominis* will not grow in an acid medium, though he was able to obtain cultures in an unsterilized neutral suspension of faeces in saline solution. Ohira and Noguchi 1917) succeeded in cultivating the oral species in diluted ascitic fluid, and Pringault (1920) states that he has obtained "some growth" of *T. hominis* in this medium. At the present time, however, it is not possible to cultivate this organism with certainty in any medium. All the attempts which we ourselves have made have been failures: but we may note that in certain liquid stools we have been able to keep *T. hominis* alive and active for periods up to a month.

It may be added that Chatton, who has successfully cultivated a *Trichomastix* from an African gecko, has recently announced that he has been able to cultivate also the *Trichomonas* of the guinea-pig. His medium consists of meat bouillon mixed with rabbit's blood. (For details see Chatton, 1920.)

(3) *Chilomastix mesnili* (Wenyon) Alexeieff, 1912.

Chief synonyms:

Cercomonas [sp. 1] Davaine, 1854.
Cercomonas hominis (A) Davaine, 1860.
Cercomonas davainei Moquin-Tandon, 1860.
"*Cercomonad* B" Cunningham, 1871.
Cercomonas intestinalis Marchand, 1875.
Trichomonas intestinalis Leuckart, 1879 (*pro parte*).
Monocercomonas hominis Grassi, 1881 (*pro parte*).
Monocercomonas hominis Epstein, 1893.
"*Trichomonas intestinalis* (Marchand)" Roos, 1893.
Macrostoma mesnili Wenyon, 1910.

* The species in the human mouth has been described by many workers—Kunstler (1888), Prowazek (1902), Goodey and Wellings (1917), etc. It is almost certainly distinct from *T. hominis* and *T. vaginalis*, and according to Goodey, differs from these in having no free flagellum at the posterior end of the undulating membrane.

Tetramitus mesnili (Wenyon) Alexeieff, 1910.
Fanapepea intestinalis Prowazek, 1911.
Difämus tunensis Gäbel, 1914.
Cyathomastix hominis Prowazek & Werner, 1914.
Chilomastix davainei (Moq.-Tand.) Kofoid, 1920.

As will be evident from the foregoing list of synonyms, this organism has long been known and has frequently been named. It appears to have been first recognizably described by Davaine, who found it in the stools of persons suffering from cholera. He first called it merely "*cercomonas*" (Davaine, 1854)—it being the first of the two different species to which he gave this name. Later (Davaine, 1860) he redescribed it, and named it "*Cercomonas hominis*, variété ou espèce A" (his variety B, as already noted on p. 65, being *Trichomonas*). From his figures and description of the organism—with its "trait longitudinal vers l'extrémité antérieure, donnant l'apparence d'un orifice *buccal ?*"—it is impossible to doubt that Davaine was dealing with *Chilomastix*.

The organism was seen again by Cunningham (1871), in India, but called by him "*Cercomonad* B"; and by Marchand (1875), who wrongly identified it with Lambl's "*Cercomonas intestinalis*" (=*Giardia*). Leuckart (1879) and other early workers confused it with *Trichomonas*, and Leuckart's "*Trichomonas intestinalis*" included both species.* Grassi's (1881*a*) species "*Monocercomonas hominis*"—to judge from some of his figures—included *Chilomastix* as well as *Trichomonas :* but the organism called "*Monocercomonas hominis* " by Epstein (1893) was undoubtedly *Chilomastix*—not *Trichomonas.* This is true also of the flagellate incorrectly named "*Trichomonas intestinalis* (Marchand)" by Roos (1893).

Wenyon (1910) recognized the present species as a form distinct from *Trichomonas*, and named it *Macrostoma mesnili.* This generic name—which had been introduced by Alexeieff—not being available, it was later proposed, by Alexeieff (1910) and others, to transfer the organism to the genus *Tetramitus.* To this genus, founded by Perty (1852), it certainly does not belong—as Alexeieff afterwards realized ; and he therefore introduced the new generic name *Chilomastix* (Alexeieff, 1912*b*)† for this species and an allied one found in Amphibia.

* Although this is not generally recognized, it is undoubtedly the case ; for Leuckart's "*T. intestinalis*" included the flagellates described by Marchand (1875), which were certainly *Chilomastix* and not *Trichomonas.*

† The name had already been tentatively suggested by this author at an earlier date (Alexeieff, 1910). A list of the synonyms of *Chilomastix* will be found on p. 87 *infra.*

In 1860 Davaine's two species of "*Cercomonas*" were renamed by Moquin-Tandon, in a work which was unfortunately overlooked until attention was recently drawn to it by Kofoid (1920). Moquin-Tandon called the species A of Davaine (= *Chilomastix*) by the new name *Cercomonas davainei*, and species B (= *Trichomonas*) *C. obliqua*. Arguing on grounds of page priority in Moquin-Tandon, and "awarding" (his own expression) *hominis* to *Trichomonas*, Kofoid therefore regards *Chilomastix davainei* as the correct name of the organism here discussed. A good case can, it is true, be made out for this combination. Nevertheless, Moquin-Tandon was not entitled to rename *both* Davaine's species; and as the application of the names which he introduced is still debatable,* we must, for the present, regard them both as doubtful. Moreover, they have never been in common use, and are unknown to the majority of workers. Consequently, we prefer, at present, to follow tradition, and refer to *Trichomonas* the species *hominis* of Davaine, while adopting Wenyon's specific name (*mesnili*) for *Chilomastix*—this being the first available if Moquin-Tandon's names are eliminated.

It remains to add that the nomenclature of this flagellate has been further complicated by the introduction of three other generic synonyms—*Fanapepea* by Prowazek (1911*a*), *Cyathomastix* by Prowazek and Werner (1914), and "*Difânus*" by Gäbel (1914). But these require no further discussion here.

Chilomastix mesnili (Pl. V, fig. 74) is one of the larger flagellates of the human intestine. It is somewhat oval or pear-shaped, but with a distinct asymmetry, and measures usually from about 10 μ to 15 μ in length; though larger and smaller individuals (ranging from some 6 μ to over 20 μ) may also be encountered. At the hind end of the body there is usually a very definite "tail," or caudal prolongation, which varies in length. The form of the body is relatively constant, and it appears rigid in comparison with a *Trichomonas*. The cytoplasm

* An even better case than Kofoid's can be made for the view that the organisms here called *Trichomonas hominis* and *Chilomastix mesnili* should really be named respectively *Trichomonas obliqua* Moquin-Tandon and *Chilomastix hominis* Davaine. It is only necessary to use the argument of page sequence consistently throughout to attain this result. Why Kofoid applies it to Moquin-Tandon and not to Davaine is not clear. From the evidence submitted by Kofoid, moreover, it seems not improbable that both Moquin-Tandon's names have priority over Davaine's. If this is really so, then the two species should be called *Chilomastix davainei* and *Trichomonas obliqua*. At present it is hardly possible to say which are the really "correct" names. (C. D.)

appears somewhat denser, and the body as a whole is invested with a thin but definite pellicle.

The finer details of structure are not easily made out, and various conflicting accounts of them have been published. We shall base our description upon our own observations,* and mention some of the discrepancies in other descriptions later.

The most striking peculiarity of the organism, when seen alive, is its large and complicated buccal apparatus. This is seen as a large and slightly spiral longitudinal cleft or groove, which extends from the anterior end for a distance of one third to one half of the length of the body (see fig. on Pl. I). Its lips are raised, and unequal. We shall call the surface on which the mouth lies, ventral; and consequently we can distinguish the two lips as right and left. The right appears the more elevated owing to the presence of a spiral groove which runs round the body outside and to the right of the lip itself. (See fig. 74, Pl. V. The right lip appears on the left of the mouth in this figure, as the organism is seen from its ventral surface.) This groove is variable in depth, being sometimes very conspicuous, and encircling the whole body, but at other times almost invisible. Its spiral course, and that of the mouth itself, is always laeotropic.

The nucleus is oval and vesicular, and lies at the anterior extremity of the body. Its membrane stains readily, and it contains a fine linin network (in fixed specimens) studded with chromatin granules of variable size. A small eccentric karyosome—sometimes more than one—is usually visible. (Cf. Pl. V, figs. 74, 75.)

At the anterior pole of the nucleus, and in close contact with its membrane, is a group of blepharoplasts. These are extremely difficult to study accurately, and to count correctly; for they are usually in contact with one another and can only be resolved in well fixed and stained specimens viewed somewhat obliquely, and with the microscope and illumination adjusted most accurately. In such individuals it can be seen that they are six in number, and arranged in a circle (fig. 76).†

* The description is based chiefly upon my observations and preparations—repeatedly checked during the last five years—and, as regards the finer details, controlled by an examination of the larger species *C. caulleryi*, which I have studied frequently since 1907. (C. D.)

† In the specimen here depicted the blepharoplasts are unusually widely separated. The specimen was selected for delineation for this reason.

They give origin to the flagella, and certain fibrils which support the lips of the mouth.

Three free and anteriorly directed flagella arise from the three dorsally situated blepharoplasts (uppermost in fig. 76). These flagella are approximately equal in length, and uniform in thickness. At the anterior end of the buccal cleft there are three ventrally situated blepharoplasts, which give origin to the following structures: (1) a long fibril supporting the right lip, and arising from the blepharoplast on the right side; (2) a shorter fibril supporting the left lip, and rooted in the left ventral blepharoplast; (3) a very slender flagellum, which lies within the mouth and arises from a blepharoplast lying between those just described. This flagellum displays a continual flickering motion in the living organism. The relations of these parts will be evident on inspection of fig. 76 (Pl. V).

The fibril supporting the right lip is longer and has a more complex course than the one in the left lip: for while the left one is short and almost straight, the right passes backwards to the posterior end of the buccal aperture, which it almost encircles, and then sinks, in a spiral course, deeply into the protoplasm (figs. 74, 76). This right fibril thus forms an incomplete loop round the hind end of the buccal groove: and it can be seen that the actual opening of the mouth, whereby food enters the body, is in this loop. The anterior part of the groove, along which the buccal flagellum lies, is merely a channel which conducts the food to the aperture at its hind end.* (The mouth opening of the organism shown in fig. 74 is in the clear area in the centre of the incomplete circle formed by the right fibre (left in figure) at the posterior border of the buccal fissure.)

Food which enters the mouth is inclosed in vacuoles in the cytoplasm, and such vacuoles are present in variable numbers in most individuals. They usually contain small bacilli and cocci, which form the chief food of this animal.

No other organs, such as an axostyle or undulating membrane, are present in this form. The tail appears to have no central skeletal support, and its protoplasm is homogeneous and free from vacuoles.

In previous descriptions of *Ch. mesnili* various discrepancies are

* These features are more clearly seen in *C. caulleryi*, but they can also be made out in *C. mesnili*. The position of the mouth opening is particularly well seen in an individual which—as sometimes happens—gets a large bacillus stuck in its mouth at the moment of ingestion.

noticeable. Most of these descriptions are, moreover, incomplete.* The most detailed accounts are those recently published by Chalmers and Pekkola (1918) and Kofoid and Swezy (1920). We may note, in the latter work, the following points. Kofoid and Swezy describe three blepharoplasts and a "centrosome," united to one another and to the nucleus by an intricate arrangement of threads. As we have already remarked, we believe there are six blepharoplasts (and no recognizable centrosome) directly attached to the nuclear membrane. The fibril supporting the right lip they call the "parabasal body," and that of the left lip the "parastyle." It is difficult to understand their grounds for thus homologizing one of these fibrils with the parabasal bodies of other flagellates; nor is it clear why the other fibril should be distinguished by a particular name. These authors also describe a "peristomal fiber" in the floor of the buccal groove, and apparently believe that it surrounds the opening of the mouth. We believe there is no such fibre, and that the mouth opening is not situated in this position. Their description of the morphology of the mouth appears to us to be undoubtedly incorrect in this and some other details. It should be noted that these authors apply the term "neuromotor system" to "the integrated fibrillar system uniting the karyosome, centrosome, blepharoplasts, flagella, and other motor organs, and the fibers of the oral region." We doubt the existence of some of these connecting fibres, and we see no advantage in thus lumping together organs possessing such different functions, and designating them by an inclusive name. There appears to us, moreover, to be little justification for applying such a term as "neuromotor" to the skeletal fibres supporting the lips of the buccal groove.

It may be noted that some observers—most recently Boeck (1921)—are of the opinion that there is an undulating membrane within the buccal groove—the buccal flagellum forming its margin. We believe this to be probably an incorrect observation, but the mistake—if it be one—is very easy to make, as the movements of this flagellum appear very like those of an undulating membrane. Prowazek (1911, 1912*b*) describes "*Fanapepea*" as often possessing only two anterior flagella.†

* Some of them—such as that of Chatterjee (1915*a*)—are so obviously imperfect that it is useless to try to discuss them.

† A subgenus *Tetrachilomastix* has been founded by da Fonseca (1916) for a similar form—not found in man—possessing 4 anterior flagella instead of 3.

We regard this also as an incorrect observation—believing this genus to be merely a synonym of *Chilomastix*. Rodenwaldt (1912) has figured what appears to be a *Chilomastix* with an axostyle, and Prowazek and Werner (1914) have named it—from his figure—*Cyathomastix*. This also, we think, is probably merely a malobservation.

Chilomastix mesnili lives in the large intestine, and possibly also in the small.* That it inhabits the large bowel is clear from the mode of its occurrence in the stools in association with other protozoa of known habitat (*e.g.*, *Giardia* and *Entamoeba coli*) in persons infected with these organisms also. It has also been demonstrated in sections of the large bowel by Wenyon (1920).

CULTIVATION. Boeck (1921) has recently succeeded in cultivating this flagellate—in association with bacteria—in a medium consisting of a modified Locke's solution and serum :† but no other worker seems to have had a similar success. By frequent subculture Boeck was able to keep a strain *in vitro* for about four and a half months, at the end of which it was accidentally lost.

MULTIPLICATION is effected by longitudinal binary fission, but stages in the process are extremely rare in the stools. A few have been noted by various workers, but no complete description of the division of this flagellate has yet been published. Boeck (1921) has recently seen various stages in his cultures, and has also observed multiple fission—into four daughter-individuals; but he has not yet given a sufficiently detailed description of these phenomena. Division has not yet been adequately studied in any other species of the genus.

The CYSTS of *Ch. mesnili* (Pl. V, fig. 77) are minute oval structures with a projection at the narrower end, which corresponds to the anterior end of the free form. Their shape may be compared with that of a lemon. (Cf. also Pl. VIII, figs. J^1, J^2, J^3.) Usually they measure 7·5 μ to 8·5 μ in length, but larger and smaller specimens may be found. When first formed they sometimes contain a lump of glycogen, of variable size (cf. Dobell and Jepps, 1917). The cyst wall is thin, colourless, and uniform in thickness, except at the pointed end, where it is slightly thickened.

* Boeck (1921) states definitely that "the habitat of the parasite is the small intestine," but he gives no evidence in support of this statement.

† The most successful medium was found to be four parts of Locke's solution (containing 0·25 per cent. dextrose—instead of the usual 0·1 per cent.) mixed with one part of human serum. The medium was alkaline (0·2 per cent. to phenolphthalein) at the beginning, and increased in alkalinity with the growth of the implanted organisms.

The cyst contains a single nucleus of relatively large size (fig. 77), which at first lies anteriorly (*i.e.*, at the pointed end) but later takes up a more central position. It is spherical, and usually shows a condensation of its chromatin at one pole, so that it has the form of a signet-ring in optical section (cf. Pl. VIII, figs. J^2, J^3). This form of the nucleus is also seen in flagellates which are about to encyst.* The other conspicuous structures within the cyst are the fibrils which support the buccal apparatus. These persist in the mature cyst and give it a very characteristic appearance. Their blepharoplasts become detached from the nuclear membrane, so that they lie freely in the cytoplasm—the entire skeletal support of the mouth lying longitudinally, in the form of an incomplete sling, in the cyst (fig. 77). The buccal flagellum can usually be made out also, lying in its normal position inside the remains of the mouth. The three blepharoplasts of the anterior flagella also persist, and can often be made out in well stained specimens (fig. 77). They become detached from the nuclear membrane at an early stage.

Neither the buccal fibrils nor the nucleus can usually be seen in the living cyst, which appears to have a homogeneous internal structure save for the presence of a few bright and very small granules (Pl. VIII, fig. J^1). These granules give some of the microchemical reactions of volutin, and probably consist of this or some allied substance. They are sometimes—but not always—visible in cysts stained with iron-haematoxylin.

Cysts of *Chilomastix* remain in this uninucleate condition for two or three weeks outside the body, if kept moist. They then begin to degenerate—the buccal structures undergoing fragmentation, and the nucleus and cytoplasm disintegrating. Like the cysts of the other intestinal protozoa, they are unable to withstand drying.

Kofoid and Swezy (1920) have described a very complicated arrangement of threads and granules within the cysts of this species. Even if their account is correct—which we do not believe—it appears to be physically impossible to prove the existence of so many structures in so small a cyst: for they figure systems of points and lines whose actual existence could hardly be demonstrated by the finest optical apparatus. They appear, however, to have no doubts themselves

* The nuclei of such individuals resemble that of the specimen shown in fig. 75 (Pl. V).

regarding their own interpretation of the optical images which they depict.* But another equally remarkable feature in their description is their account of nuclear division within the cyst. They describe and figure a mitosis of the nucleus, and the formation of two daughter nuclei : and they even say that "it is probable that one or two other nuclear divisions follow in the cysts, though we have not been able as yet to find them." We can find no justification whatsoever for such a statement, and we are completely at a loss to account even for the single nuclear division which they describe. Although we have kept cysts for many weeks, in varying conditions, until they finally degenerated and died, and although we have examined thousands upon thousands of cysts in human faeces, we have never yet seen a single cyst containing more than one nucleus. If nuclear division does occur within the cyst, it must be excessively rare.

Finally, it should be noted that the 4-nucleate cysts doubtfully attributed to this species by Wenyon (1915) were in reality those of *Endolimax nana*, as he showed subsequently (Wenyon and O'Connor, 1917); whilst those of Swellengrebel (1917) really belong to *E. histolytica*. On the other hand, Lynch (1916) really observed the cysts of *Chilomastix*, but wrongly referred them to *Trichomonas*. The cysts were overlooked by most of the earlier workers who observed *Chilomastix*, and were first carefully described by Wenyon and O'Connor (1917) and Dobell and Jepps (1917), though well known to these and many other English workers for some time previously.

(4) *EMBADOMONAS INTESTINALIS* Wenyon & O'Connor, 1917, *emend.*

Synonym :

Waskia intestinalis Wenyon & O'Connor, 1917.

This little flagellate was found by Wenyon and O'Connor (1917) in Egypt, and was placed originally in a new genus—*Waskia*. It should be

* Some of the details of structure which they depict in the cysts of this organism are undoubtedly incorrect. I may say that I first found these cysts in the winter of 1915-16, before they had been described, and I then identified them and studied them in detail. My pupils and fellow-workers who studied them at the same time readily confirmed my observations, and in the course of several years not one of them succeeded in finding a single cyst containing more than one nucleus though search was constantly made for such specimens. (C. D.)

referred, however, to the genus *Embadomonas* Mackinnon, 1911 (emend. 1915),* as Chalmers and Pekkola (1918) and others have pointed out.

E. intestinalis (Pl. V, fig. 72) is a minute and more or less ovoid flagellate, measuring some 5-6 μ in length by 3-4 μ in breadth. It possesses a single anteriorly placed nucleus, with a small central karyosome or a few rather indefinite chromatin granules. Immediately behind the nucleus, the body has a large and elongated depression—the mouth. This is supported round its edges by fibres, as in *Chilomastix*. On the surface of the nuclear membrane lying towards the mouth there are two blepharoplasts, which give origin to two flagella. One of these is long and thin, and projects anteriorly. The other is shorter and thicker, and arises just behind it, lying, during life, partly within the mouth. By means of its two flagella the animal performs characteristic movements. The long anterior flagellum serves for progression : the short one moves more slowly, and independently, and causes a jerky movement of the organism as a whole.

Food, consisting of minute bacilli and cocci, is taken in through the mouth, and is then seen to lie in tiny vacuoles in the cytoplasm. There is no permanent anus, and no axostyle or undulating membrane can be made out.

The structural details of this flagellate are excessively difficult to determine, owing to its extremely small size. It is probable, however, that its structure is almost identical with that of the larger species of the genus (from insects), excellently described by Mackinnon (1915).

A few stages of MULTIPLICATION, by longitudinal fission, have been observed by Wenyon and O'Connor (1917) ; but the process has not been made out in every detail.

The CYSTS are very minute somewhat pear-shaped structures (Pl. V, fig. 73). They measure 4·5 μ to 6 μ in length,† and resemble those of *Chilomastix*—though of course they are considerably smaller. They are uninucleate, and contain a long deeply-staining looped thread, which

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* From a careful examination of specimens of the type species, kindly given to me by Dr. Mackinnon, and from a study of some of Captain O'Connor's original preparations of "*Waskia*," I consider that there is no room for doubt on this question. It may be noted, however, that da Fonseca (1920) maintains that *Embadomonas* and *Waskia* are distinct genera, though he does not give any cogent reasons for this view. (C. D.)

† These figures are given by Wenyon and O'Connor (1917) for living cysts. According to my measurements (stained specimens) the length of the cysts is 4-4·5 μ, their breadth approximately 3 μ. Their shape is most aptly compared, I think, with that of a grape-seed. (C. D.)

is evidently—as in *Chilomastix*—the remains of the fibril supporting the margin of the mouth in the free flagellate.*

It is still uncertain what part of the bowel this flagellate inhabits. It is very small and difficult to study, and appears to be very rare. A similar species has recently been described from the caecum of a Brazilian monkey (*Cebus caraya*) by da Fonseca (1917). For this species he proposes the name "*Waskia*" *wenyoni*. It measures 14 μ by 12 μ, and is therefore considerably larger than *E. intestinalis*. It should be added that the "dividing" forms seen by Wenyon and O'Connor (1917) appear to be regarded by this author not as stages in division, but as the normal forms of the animal. "*Waskia*" *wenyoni* is thus said to have "two cytostomes" and a double set of flagella.† It is not easy to comprehend Fonseca's reasons for adopting such an interpretation.

(5) *ENTEROMONAS HOMINIS* da Fonseca, 1915, *emend.*

Synonyms:

? *Octomitus hominis* Chalmers & Pekkola, 1916.
Tricercomonas intestinalis Wenyon & O'Connor, 1917.
"*Monocercomonas*" Chatterjee, 1917.
Trichomastix hominis Chatterjee, 1917.
Dicercomonas soudanensis Chalmers & Pekkola, 1919.
Diplocercomonas soudanensis Chalmers & Pekkola, 1919.
Enteromonas Bengalensis Chatterjee, 1919.

The organism, or organisms, to be noticed in this section must be regarded as still somewhat problematic: and the views here put forward‡ are tentative—further investigations being necessary to establish their correctness (or error).

In 1915 da Fonseca described a new flagellate found by him in

* Wenyon and O'Connor (1917) describe the nucleus as becoming drawn out in the cysts. The above is the interpretation of the appearances which I believe to be correct —after studying these cysts with great care. Details are, however, extremely difficult to determine accurately. (C. D.)

† See especially the recent "redescription" of "*Waskia*" by da Fonseca (1920).

‡ For this section, and the opinions expressed in it, I alone am responsible. Up to the time when Captain O'Connor left England, we had been unable to come to any definite conclusions concerning the identity or diversity of the various flagellates here discussed. I have now been compelled to write this section entirely by myself, and with insufficient material at my disposal to settle some of the disputed points. If my judgement is at fault, it is only fair to point out that my collaborator is in no way to blame. (C. D.)

human faeces, in Brazil, and named it *Enteromonas hominis*. He has since published several redescriptions of it (da Fonseca, 1916, 1918, 1920). According to his latest observations (1920) the flagellate—which is very small—possesses three anterior flagella. Two of these are short, and directed forwards; the third is longer, and recurrent, but not attached to the surface of the body. Fonseca has not yet described the cysts of this organism.

Wenyon and O'Connor (1917) and O'Connor (1919) found a similar flagellate in Egypt, and named it *Tricercomonas intestinalis*. It appears, however, to differ from Fonseca's form in having four flagella—three (not two) directed forwards, and the posteriorly-directed one adherent to the surface of the body. The cysts of this flagellate were, moreover, described and figured (Wenyon and O'Connor (1917), Pl. III).

Chalmers and Pekkola (1917, 1917*a*, 1918), later found—also in Egypt—an organism believed by them to be identical with that described by Fonseca. They added practically nothing worthy of note to his observations except the statement that the three anterior flagella are all equally long and all directed forwards.

An apparently similar flagellate was found in India by Chatterjee (1917), who first named it "*Monocercomonas*," and shortly afterwards *Trichomastix hominis* (1917*a*). From his confused account of this organism*—obviously based, in part at least, upon inexact observations—it appears to differ from *Tricercomonas* chiefly in that the trailing flagellum is not attached to the body, whilst "in some specimens only two anteriorly directed flagella are seen and one posteriorly directed." No cysts were described, and it seems clear that the organism is not, in any case, a *Monocercomonas* or *Trichomastix* (= *Eutrichomastix*). The same author has more recently (Chatterjee, 1919) described a "new" species of "*Enteromonas*," from another case, and named it *E. bengalensis*. It is impossible, however, from the figures and description, to identify this organism with certainty.

Leger (1918) believes he has seen Fonseca's *Enteromonas* in French Guiana. He saw only two anteriorly directed flagella on his organisms, but notes that in one individual they seemed to be doubled in number. The posterior flagellum, according to his account, "se porte en arrière

* The two accounts appear to me to relate to the same organism, but I am not certain whether this is the author's own view. His papers are hard to understand, and bristle with misspelled names, misquotations, and other unfortunate errors. (C. D.)

le long du corps, sans être cependant réuni à celui-ci par une membrane ondulante." (It is not clear from this whether or no it is adherent to the body.) Cysts are not described.

Chalmers and Pekkola (1919) have more recently described, again from Egypt, another "new" flagellate, apparently identical with *Tricercomonas* save in that it possesses two anteriorly directed flagella instead of three. Its cysts were not described. At first this organism was called *Dicercomonas soudanensis*, but its generic name was subsequently changed to *Diplocercomonas* (Chalmers and Pekkola, 1919*a*)—the first name not being available.

It appears not improbable that all these different descriptions really refer to one and the same species. Allowance must be made for the fact that all the workers—Wenyon and O'Connor excepted—have studied a very small amount of material, and it must be remembered that the organisms in question are all extremely minute, and admittedly difficult to study. No adequate cytological descriptions have yet been given of any of them, and the published figures are obviously, in many cases, inaccurate (*e.g.*, as regards the insertion of the flagella). It seems far more probable that some of the descriptions are incorrect, than that six species, belonging to five distinct genera, really exist—all so much alike, and differing only in such comparatively trivial points as have been described.* Tentatively, therefore, we include all these flagellates in one species, which, by the rule of priority, must be called *Enteromonas hominis* da Fonseca.

The apparent discrepancies in the published descriptions seem to be capable of simple explanations. It may be taken that Wenyon and O'Connor's account of "*Tricercomonas*" is essentially correct, and that this organism has usually three free anterior flagella—only two of which can sometimes be made out in stained preparations ("*Diplocercomonas*" of Chalmers and Pekkola). Sometimes the attachment of the posteriorly directed flagellum to the body is incomplete, or has not been made out (da Fonseca's "*Enteromonas*"), or the posterior flagellum has been

* I have been led to this view partly from reading the published descriptions, and partly as a result of studying some of Capt. O'Connor's original preparations of "*Tricercomonas*." I have also been able to study preparations of (apparently) the same organism given to me by Miss M. W. Jepps, who encountered it in a military patient at Southampton. In these preparations all the flagellar arrangements described by different workers may be seen in different specimens, but their interpretation is often a matter of great difficulty. I attach particular importance, therefore, to the observations made by Wenyon and O'Connor on the living animals. (C. D.)

entirely overlooked (Chalmers and Pekkola's "*Enteromonas*"). The organisms described by Chatterjee and Leger might be—from the incomplete accounts—either "*Tricercomonas*" or "*Enteromonas*." There are no sufficient characters given to distinguish them from either.

It appears somewhat significant that the describers of "*Enteromonas*" have varied their descriptions from time to time. At all events, the circumstance appears to favour the conclusion here reached. In his earlier accounts, Fonseca (1915, 1916) stated that "*Enteromonas*" possesses 3 anterior flagella—2 short ones directed forwards, and a longer one trailed behind. After the appearance of Chalmers and Pekkola's papers—in which these authors stated that there were 3 flagella of equal length, all directed forwards—Fonseca (1918, 1918*a*) came to the conclusion that they were right: "none of the three flagella," he writes, "is constantly recurrent, all three are habitually directed forwards." But now (Fonseca, 1920) he reverts to his original account, and describes and figures the organism with 2 flagella directed forwards and a longer one trailing behind. Meantime Chalmers and Pekkola have found similar forms, with the posterior flagellum adherent to the body, but have referred them to the new genus "*Diplocercomonas*." It thus seems highly probable that the real arrangement is that described in "*Tricercomonas*," and the other accounts rest upon inexact or incomplete observations.

We draw these conclusions with some hesitancy, and believe it possible—but not probable—that two distinct organisms are included in the genus *Enteromonas* as here constituted: (1) *Enteromonas* as defined by Fonseca (1920), with 2 anteriorly directed flagella, and one posteriorly directed and free; and (2) *Tricercomonas*, as defined by Wenyon and O'Connor (1917), with 3 free anterior flagella and a longer posteriorly directed one adherent to the surface of the body. Further investigations alone can determine these points—the discovery of the cysts of the flagellates described by da Fonseca, Chatterjee, and Chalmers and Pekkola, being especially desirable.

Enteromonas hominis is a very small oval or rounded flagellate, of somewhat changeable shape, measuring usually 4μ to 8μ in length when alive. Stained specimens measure somewhat less. The flagellate (Pl. V, figs. 62, 65) possesses a single vesicular nucleus, situated at the anterior end and containing a large central karyosome. The nucleus

is more or less drawn out at its anterior pole, and at this point there are at least two (probably more) minute blepharoplasts (fig. 65), which give origin to the four flagella. These are approximately equal in length. Three of them are free, and directed forwards : the fourth, which may be slightly longer, is directed backwards. It passes over the surface of the body, to which it is adherent (? always), and terminates freely at the hind end, or sometimes laterally (figs. 62, 65). The cytoplasm contains food vacuoles, inclosing ingested bacteria. There is no permanent mouth, however, and no axostyle, no undulating membrane, or other conspicuous organ.

The details of structure are extremely difficult to make out with precision, owing to their minute dimensions. The figures (Pl. V, figs. 62-65) here given were all drawn from specimens in the same preparation,* and the apparent variations which they exhibit, from the typical structure just described, are adduced in support of the interpretation of the genus here advanced. In figs. 62 and 65 we see the typical form, with its full complement and typical arrangement of flagella. Fig. 63 shows an individual in which the posteriorly directed adherent flagellum is not visible—either because it is unstained or because it has become detached. This is a form corresponding with Fonseca's *Enteromonas*. The individual shown in fig. 64 possesses apparently only two anterior flagella instead of three. This is the type of organism named *Diplocercomonas* by Chalmers and Pekkola. It appears highly probable that all these organisms, obtained simultaneously from the same patient, belong to the same species, and that the differences in structure are more apparent than real.

MULTIPLICATION by longitudinal fission has been observed by Wenyon and O'Connor (1917), who have figured a few stages. We have at present insufficient material at our disposal to describe the process in detail.

The CYSTS of this species have also been described by Wenyon and O'Connor (1917). They are elongate oval structures, measuring 6-8 μ in length by 3-4 μ in breadth.† When first formed (fig. 66) they con-

* The preparation is one of Capt. O'Connor's original slides containing the type species of Wenyon and O'Connor's genus "*Tricercomonas*." I have restained it, and now preserve it as a type specimen of the genus *Enteromonas* as here defined. (C. D.)

† According to my measurements (of stained cysts—in which the walls are usually clearly discernible), the correct dimensions of the cysts are 6-6·5 μ by 4-4·5 μ. The

tain a single nucleus. This subsequently divides into two (fig. 67); and each of these daughter nuclei again divides, so that the mature cyst is 4-nucleate (fig. 68). The nuclei at all stages occupy a characteristic position at the poles of the cyst. Small deeply stainable blocks or rodlets of "chromatoid" substance are sometimes present in the cysts of this species—usually at the ends, near or around the nuclei.

It is still uncertain what part of the intestine this flagellate inhabits. Its geographical distribution appears to be wide: and it may be noted that Fonseca (1918*a*) has recently described another species of *Enteromonas* from the rabbit, in Brazil. Some of the describers of *E. hominis* regard it as pathogenic, but their evidence seems very questionable.

In conclusion, we may refer to the curious flagellate described under the name "*Octomitus hominis*" by Chalmers and Pekkola (1916). This organism appears to resemble a "*Tricercomonas*" with two sets of flagellar organs. From the description and figures it is probably not an *Octomitus*, as it possesses only one nucleus (?) and blepharoplast (?). We suggest that it may be a dividing form of "*Tricercomonas*" (*i.e.*, *Enteromonas*, as here defined): and we may note—as it may throw some light on this form—that Fonseca (1920) states that he has observed "multiflagellate" individuals of *Enteromonas hominis*. Further information about this remarkable "*Octomitus*" is much needed.

Synonymy and Homonymy of the Genera of Flagellates occurring in the Human Intestine.

In the foregoing descriptions of the intestinal flagellates of man it has frequently been necessary to refer to their genera. Some of these are still in a condition of great confusion, owing to wrong identifications, misapplication of names, the introduction of new names for forms already named, and the re-introduction of designations already preoccupied or abolished. We propose, therefore, to recapitulate and amplify what has already been said in this chapter, concerning the generic names of the forms in question, in the following tables, which we offer for the use of students of the group. These tables, in conjunction with the references to original works given at the end of the

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measurements given above are those of Wenyon and O'Connor. In stained preparations the cysts are smaller than those of *Endolimax nana*, which they otherwise resemble somewhat. (C. D.)

book, will, it is hoped, enable the reader to obtain a clear and correct conception of the nomenclature and systematics of this confused and somewhat difficult group of organisms.*

Genus 1. *GIARDIA* Kunstler, 1882, *emend.* Alexeieff, 1914.

Synonyms :

Cercomonas Lambl, 1859.
[nec *Cercomonas* Dujardin, 1841.]
Hexamita Davaine, 1875 (pro parte).
[nec *Hexamita* Dujardin, 1841.]
Dicercomonas subgen. *Dimorphus* Grassi, 1879.
[nec *Dicercomonas* Diesing, 1865.]
[nec *Dimorphus* Haller, 1878.]
Megastoma Grassi, 1881.
[nec *Megastoma* Swainson, 1837, et al.]
Lamblia Blanchard, 1888.

Genus 2. *TRICHOMONAS* Donné, 1837, *emend.* Ehrenberg, 1838.†

Synonyms :

Trico-monas Donné, 1837.
Cercomonas (pro parte) Davaine, 1854, 1860.
[nec *Cercomonas* Dujardin, 1841.]
Saenolophus Leuckart, 1863.
Monocercomonas Grassi, 1879.
Cimaenomonas Grassi, 1881.

Including the "subgenera" *Tetratrichomonas* Parisi, 1910 ; *Pentatrichomonas* Mesnil, 1914 (? Chatterjee, 1915) = *Hexamastix* Derrieu & Raynaud, 1914 [nec Alexeieff, 1912] ; and *Tritrichomonas* Kofoid (1920).

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* For these tables I alone am responsible. I have made every effort to make them as correct as possible, and I hope that, brief though they be, they contain everything of importance to the systematist. (C. D.)

† I am not certain whether this name should not really be accredited to Dujardin. He suggested it to Donné, who misspelled it ; but Dujardin (1841) himself gave it correctly in his own work. (C. D.)

Genus 3. *CHILOMASTIX* Alexeieff, 1910.

Synonyms:

Cercomonas (pro parte) Davaine, 1854, 1860.
[nec *Cercomonas* Dujardin, 1841.]
Trichomonas (pro parte) Leuckart, 1879, et al.
[nec *Trichomonas* Donné 1837, emend.]
Monocercomonas Epstein, 1893.
[nec *Monocercomonas* Grassi, 1879.]
Macrostoma Alexeieff, 1909.
[nec *Macrostoma* Latreille, 1825, et al.]
Tetramitus Alexeieff, 1910.
[nec *Tetramitus* Perty, 1852.]
Fanapepea Prowazek, 1911.
Difämus Gäbel, 1914.
Cyathomastix Prowazek & Werner, 1914.

Genus 4. *EMBADOMONAS* Mackinnon, 1911, *emend.* 1915.

Synonyms:

? *Fanapepea* (pro parte) Prowazek, 1911.*
Waskia Wenyon & O'Connor, 1917.

Genus 5. *ENTEROMONAS* da Fonseca, 1915, *emend.*

Synonyms:

Tricercomonas Wenyon & O'Connor, 1917.
Monocercomonas Chatterjee, 1917.
[nec *Monocercomonas* Grassi, 1879.]
[nec *Monocercomonas* Epstein, 1893.]
Trichomastix Chatterjee, 1917.
[nec *Trichomastix* Vollenhoven, 1878.]
[nec *Trichomastix* Blochmann, 1884, = *Eutrichomastix* Kofoid & Swezy, 1915.]
Dicercomonas Chalmers & Pekkola, 1919.
[nec *Dicercomonas* Diesing, 1865.]
[nec *Dicercomonas* Grassi, 1879.]
Diplocercomonas Chalmers & Pekkola, 1919.

* It seems to me possible that some of the smallest forms placed in this genus by Prowazek (1911*a*) should be referred to *Embadomonas* rather than to *Chilomastix*. (C. D.)

Key for Determination of Genera and Species.

We give below a key for the identification of the flagellates found in the human intestine. The characters used are those of the free flagellates and also of their cysts—since the latter are of importance for distinguishing some species from similar ones which occur in other hosts.

1. (*a*) Free flagellate with 2 flagella ... Genus *Embadomonas* 2.
   (*b*) „ „ „ 3-6 flagella ... ... 3.
   (*c*) „ „ „ 8 flagella ... Genus *Giardia* 8.
2. Very small (5-6 μ); flagella anterior, unequal; mouth large; cysts piriform, 4·5-6 μ long ... ... *E. intestinalis.*
3. (*a*) With an axostyle and undulating membrane ... ... Genus *Trichomonas* 4.
   (*b*) With neither axostyle nor undulating membrane ... ... 5.
4. With 3-5 free anterior flagella, and a posteriorly directed one forming the margin of the undulating membrane; cysts unknown ... ... *T. hominis.**
5. (*a*) With 3 free anterior flagella, and a fourth smaller one lying within the large mouth ... ... Genus *Chilomastix* 6.
   (*b*) With 3 (or 2 ?) anterior free flagella, and one posteriorly directed and more or less adherent to surface of body ... Genus *Enteromonas* 7.
6. Length ca. 7-20 μ; cysts 7-9 μ long, lemon-shaped .. .. *C. mesnili.*
7. Very small (4-8 μ); cysts elongate oval, 4-nucleate, 6-8 μ long ... ... *E. hominis.*
8. Bilaterally symmetrical, with large anterior ventral sucker; 2 nuclei, 2 axostyles, and flagella in 4 pairs; cysts oval, ca. 12 μ long ... ... *G. intestinalis.*

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* The "subgenera" (see p. 68) found in man are:
(1) *Trichomonas* (= *Tetratrichomonas*), with 4 free anterior flagella.
(2) *Tritrichomonas*, with 3 free anterior flagella.
(3) *Pentatrichomonas*, „ 5 „ „ „

Intestinal "Flagellosis."

The condition of being infected with intestinal flagellates is sometimes termed "Flagellosis," though various other designations have also been used.* By some workers the condition is regarded as more or less pathological, and "flagellosis" is thus considered to be a disease. We do not share this view, and regard it as almost certain that intestinal flagellates are usually harmless to their hosts. It is impossible to discuss here all the facts, and all the inferences drawn from them, which have been adduced by those who regard the intestinal flagellates of man as pathogenic: but we shall note the chief points, and attempt to give some justification for our opinions.

Much confusion has, undoubtedly, arisen in the past owing to the indiscriminate use of the word "parasite." There is a natural tendency to regard all "parasites" as harmful; and intestinal flagellates, being regarded as "parasites," are consequently suspect. But it should be remembered that the intestinal flagellates of man, and of most other animals, are not parasitic in the strict sense of the term: they are more properly called commensal.† It is, indeed, still very doubtful whether any truly parasitic intestinal flagellates exist—in any host. In the vast majority of cases, at any rate, no harmful effects due to their presence can be demonstrated.

Again, it is to be remembered that the frequent finding of flagellates in the stools of persons suffering from diarrhoea or dysentery does not in any way incriminate these organisms as "causes" of the disorders observed. Flagellates are, it is true, found more often in persons with diarrhoea than in healthy persons—but for the simple reason that the stools of healthy persons are seldom examined: and careful examination of the stools of healthy people has shown that they are probably infected with flagellates quite as frequently as patients suffering from diarrhoeic disorders.

If a person naturally infected with intestinal flagellates happens to suffer from diarrhoea, examination of his stools will then usually reveal their presence: but if his stools are normal, only the cysts of these flagellates will, from time to time, be discoverable in them—not the active forms. Consequently, the circumstance that *active* flagellates are not usually found in the stools of healthy people does not imply that such people are not commonly infected. An attack of diarrhoea often

* Cf. Introduction, p. 15. † Cf. Introduction, p. 13.

leads to the discovery of flagellates in a person not previously suspected of harbouring them: and it is probably true that the so-called "flagellate diarrhoeas" are not diarrhoeas "caused" by flagellates, but diarrhoeas which have "caused" the flagellates to make their appearance in the stools.

We sometimes read of cases of "flagellate diarrhoea" in which a "cure" has been effected by some form of treatment. This or that drug was administered, and the diarrhoea ceased: the flagellates then disappeared from the stools. Evidence of this sort is then considered to corroborate the belief in the pathogenicity of the flagellates concerned. All such cases, however, have a very different complexion when carefully examined. All the so-called "specifics" for flagellate infection hitherto advocated are probably without action upon these organisms: at all events, nobody has yet produced any good evidence to show that any drug whatever can eradicate an infection with intestinal flagellates.* It appears highly probable that all the "cures" which have been claimed are based upon insufficient examination of the stools after treatment. More prolonged examination would have shown that the flagellates were still present in these "cured" cases.† Consequently, if a cure of the clinical condition was effected, without removing the flagellates, the evidence really indicates that the flagellates were not causally concerned in the production of the disorder.

Often, too, we read that some intestinal flagellate was the "cause" of a patient's intestinal disorder because "no other cause could be found." The absurdity of such statements is obvious. If such reasoning were permissible, one would have to suppose that many cases of diarrhoea, in which neither flagellates nor other organisms can be found, are due to no cause at all.

Evidence has been adduced to show that some of the intestinal flagellates are capable of invading the tissues, and causing definite lesions in the bowel. This evidence has recently been reviewed by Haughwout (1918), and most of it is highly unconvincing. Perhaps the strongest evidence is that just brought forward by Wenyon (1920), who has found *Trichomonas* present in the wall of the bowel: but here, as in all such cases, it remains doubtful whether the invasion of the tissues—which, in Wenyon's case, appeared otherwise normal—occurred

* Cf. p. 159.

† I have examined many such cases, and always with the same result. (C. D.)

before or after the death of the patient. The evidence in other cases also requires further explanation. Biland (1905), for example, has found peculiar lesions in the intestine of a patient infected with *Trichomonas*. But the lesions were in the small intestine and no flagellates were found in them, whilst the normal habitat of this flagellate is the large intestine. Again, in the often-quoted case of Fairise and Jannin (1913), the ulceration, believed by them to have been caused by *Giardia*, was found in the large bowel; whereas this flagellate lives in the small intestine. Cysts of *Giardia* were found in the ulcers, moreover,—a very remarkable phenomenon. It is clear that such findings themselves require to be explained. They are not, as they stand, easily intelligible; and they are far too ambiguous to be used, at present, as evidence of the pathogenicity of the intestinal flagellates.

Trichomonas hominis has occasionally been observed to ingest red blood-corpuscles, and this has been regarded by some observers as evidence of its pathogenicity (cf. Woodcock, 1917; Haughwout and de Leon, 1919). It is clear, however, that the mere fact that a flagellate is able to eat a blood-corpuscle, when presented to it, supplies no evidence whatever that the flagellate itself attacks the tissues or has been in any way responsible for the appearance of the corpuscles in the stools.* Haughwout and de Leon (1919) could find no other likely cause for their patient's dysentery than the numerous "*Pentatrichomonas*" in her stools: and they observed many of the flagellates, in the bloody mucous stools, containing ingested red corpuscles. But in similar cases which one of us has studied (O'Connor, 1919), careful investigation showed that the patients were suffering in reality from bacillary or bilharzial dysentery—which was sufficient to account for the corpuscles present in the stools. The presence of trichomonads concomitantly, and the fact that they were ingesting red corpuscles, can hardly, in such cases, be regarded as evidence of the pathogenicity of these flagellates. The obvious conclusion to draw is that *Trichomonas* will sometimes eat blood-corpuscles when they happen to be available: any further inference appears unwarranted.

Among the older observers opinion was divided as to the pathogenicity of the intestinal flagellates. Grassi (1888) and others regarded them as harmless. Most physicians, however, considered them—and

* It may be noted here that the amoeba of the frog (*Entamoeba ranarum*), as I have shown elsewhere (Dobell, 1909), will ingest red blood-corpuscles when these happen to be present in the gut contents. It is certain, in this case, that the amoeba is not pathogenic, and does not attack the tissues. (C. D.)

still consider them—harmful to a greater or less extent. Among recent workers who have endeavoured to incriminate one or other of the intestinal flagellates* as "causes" of human disease, may be mentioned Brumpt (1912), Nattan-Larrier (1912), Mello-Leitão (1913), Escomel (1913, 1919), Mathis (1914), Gäbel (1914), Lynch (1915*a*), Rhamy and Metts (1916), Kennedy and Rosewarne (1916), Chatterjee (1917), Sangiorgi (1918*a*), Labbé (1919), Boeck (1921). We believe that few who read these and similar recent papers in a critical spirit will find any conclusive evidence of the pathogenicity of the flagellates concerned. It is impossible, however, to discuss all these works in detail here, and we must therefore be content, after briefly expressing our own view of the facts, with referring the reader interested in the subject to the foregoing papers and others to which these will lead him.

There is one other point which must be mentioned before concluding. This is the debated question as to whether the intestinal flagellates found in man occur also in other animals. It is often stated that man acquires his infection from some other host: for example, *Giardia* and *Trichomonas* are known to occur in rodents, and it has been assumed that these animals act as "reservoirs" of human infection.

Stated briefly, the facts appear to be as follows. Species of *Giardia* occur in rats, mice, guinea-pigs, rabbits, and other rodents, and also in the cat.† At present it is not certain whether these are of the same or of different species, nor whether any or all of them belong to the species found in man (*G. intestinalis* Lambl). They are all very much alike, though some authors (*e.g.*, Bensen, 1908) believe that they are distinguishable. Attempts have been made by many workers (*e.g.*, Perroncito (1888), Russell (1916), Fantham and Porter (1916), etc.) to infect rodents with *G. intestinalis* by feeding them upon cysts from human stools, and in most of such experiments success has been claimed. The evidence is, however, still far from conclusive, since the animals used for such experiments all belong to species which are themselves very commonly infected in nature with forms of *Giardia*

* It is, perhaps, a mistake to discuss flagellates in general as possible "causes" o diarrhoea: for it is possible that some of them are harmless, others harmful. At present, however, there appear to be no sound reasons for discriminating between the species in this way. It seems to us, on the other hand, to be clearly unjustifiable to discuss simultaneously whether the flagellates *and ciliates* are pathogenic—as has been done recently by Haughwout (1918): for the case against *Balantidium* has been clearly made out, but the pathogenicity of such an organism in no way incriminates protozoa belonging to quite a different group.

† This was first shown by Grassi (1881). The form in the rabbit had been previously seen, and named *Hexamita duodenalis*, by Davaine (1875).

not certainly distinguishable from *G. intestinalis*. It is extremely difficult to obtain "clean" animals for such experiments, and the evidence so far produced cannot, therefore, be regarded as supplying a definite proof that *G. intestinalis* can be transmitted to any laboratory animal. The same objections apply, *mutatis mutandis*, to the experiments which have been carried out with *Trichomonas* and *Chilomastix*. The confidence of the experimenters in the success of their experiments can hardly counterbalance the obvious deficiencies in their controls.

Moreover, it seems hardly necessary to regard rats and mice, or any other animals, as "reservoirs" of human flagellate infections. Such a conception was, no doubt, plausible in the days when these flagellates were believed to be uncommon in man, and, in this host, productive of disease : but now that we know that healthy people everywhere are very frequently infected with *Giardia* and other flagellates, it seems unnecessary to look for "reservoirs" outside the human species itself. Indeed, experiments such as those of Fantham and Porter (1916) appear to prove too much. They claim to have shown that laboratory animals can be experimentally infected with *Giardia intestinalis*, and that it is definitely pathogenic to these animals. But the experiments appear to show that the *Giardia* of man is not merely pathogenic but frequently fatal to mice and other animals. If this is so, it is hardly possible to reconcile the fact with the contention that mice act as a "reservoir" of human *Giardia* infection. We know, too, that mice are almost always infected with *Giardia* in nature, without any harmful results being demonstrable. It thus appears impossible to accept all these conclusions.

At the present time it seems to us best to regard the flagellates of rodents as belonging to species which are distinct from those occurring in man, but the question can be answered only by further observations and experiments.

We may, in conclusion, sum up this section by saying that, in our opinion, there is as yet no good evidence to prove that any intestinal flagellate found in man is pathogenic, but that there is very considerable evidence to show that most and probably all of them are harmless. Moreover, the evidence that the species found in man are identical with those of other hosts is inconclusive, and there is at present no good reason to suppose that any host but man can or does act as a natural "reservoir" of human infection.

CHAPTER V.

THE INTESTINAL COCCIDIA OF MAN. COCCIDIOSIS.

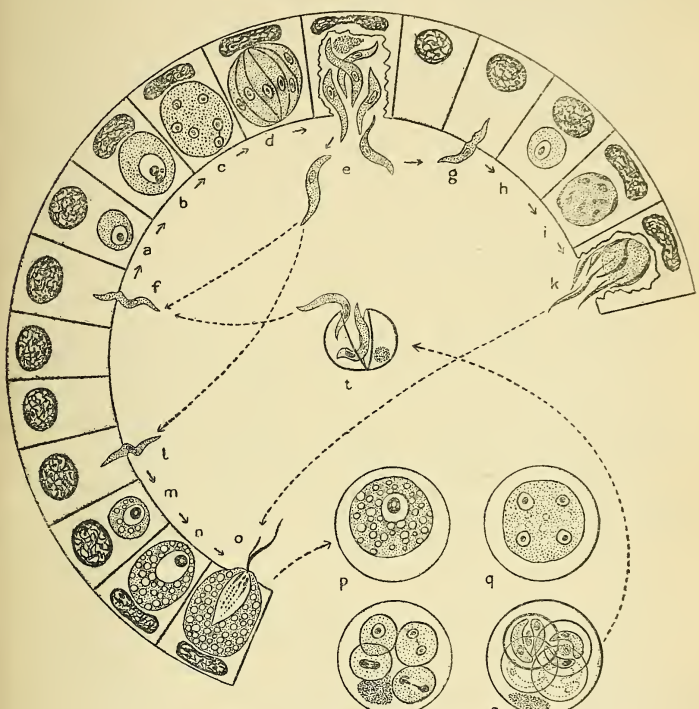
It has been noted already in the Introduction (p. 5), that the Phylum of the Protozoa called Sporozoa is represented in the human bowel by several species belonging to the Class Coccidia. These will be briefly described in the present chapter.

The Sporozoa form a very large group of exclusively parasitic protozoa. If we leave out of account the so-called "Neosporidia"—a group whose inclusion is, nowadays, more than questionable—then the Sporozoa may be said to consist of three closely related classes, forming a well-defined and "natural" group. These are (1) the Haemosporidia —containing the malarial parasites and their allies; (2) the Coccidia; (3) the Gregarinida—a group of parasites occurring in invertebrates only. The first two are so intimately related to one another that they are commonly included in one group—the Coccidiomorpha (Doflein).

The Coccidia themselves form a very homogeneous group, with a characteristic life-cycle of some complexity. In its general outline this life-cycle is closely similar to that of the malarial parasites; though a coccidium usually, but not always, passes the whole of its life in a single host—not, like a malarial parasite, in two hosts. Unfortunately, the complete life-cycle has not yet been worked out for a single one of the intestinal coccidia of man. Only fragments of their life-histories are at present known. In order that the known stages may be correctly understood, therefore, we must preface our description of them with a short account of the development of a typical member of the Coccidia. This will be readily followed with the aid of the accompanying diagram (Text-fig. B).

The young coccidium (*a*) is a minute and usually oval organism, living within a cell—usually epithelial—of its host. (In the figure, the cells are supposed to be those of the epithelium lining the gut—as seen in a diagrammatic partial cross-section.) The little parasite contains a single nucleus, probably of a complicated structure. The young

organism soon grows, at the expense of the host-cell, into a large, plump, asexual individual or SCHIZONT (*b*). It then reproduces by a process of multiple fission, or SCHIZOGONY—its nucleus first dividing repeatedly (*c*), and its cytoplasm then splitting (*d*) to form as many vermiform young as there were nuclei. These young forms are called



TEXT-FIG. B.
Diagram illustrating the Life-history of a Coccidium.

MEROZOITES (or schizozoites), and in their formation a small portion of the parent schizont is usually left over as a residual body, which ultimately dies and disintegrates. The merozoites are actively motile. They wriggle out of their host-cell (*e*), and soon find a new one, which they promptly invade (*f*). Having bored into it, they round themselves off and cease to move (*a*), and then begin the process of schizogonic

development anew (*a-f*). The events just described constitute the ASEXUAL part of the life-cycle.

Sooner or later, schizogony ceases, and a SEXUAL cycle is initiated. The merozoites (*e*), instead of becoming schizonts once more, develop into MALE and FEMALE individuals (so-called "microgametocytes" and "macrogametocytes"). In the males (*g*, *h*), the nucleus multiplies (*i*) and finally gives rise to a brood of MICROGAMETES, which are usually flagellate, and which break away from the body of the male and swim off (*k*) in search of macrogametes. The females (*l*, *m*) do not form broods of gametes, but each becomes converted, as a whole, into a single MACROGAMETE (*n*). When a microgamete encounters a macrogamete, it penetrates it at one pole—ultimately entering its nucleus (*o*). This process of FERTILIZATION—which is accompanied by very complex nuclear changes—results in the formation of a uninucleate ZYGOTE, which, in the case of intestinal coccidia, usually falls into the lumen of the gut (*p*). It may be noted that there is probably no reduction of the chromosomes during the formation of the gametes—the so-called "nuclear reduction" occurring at these stages being merely a fragmentation of the karyosome of the sexual individual. The chromosome cycle in the Sporozoa is peculiar, and reduction occurs immediately after—not before—fertilization (Dobell and Jameson, 1915).

The zygote (*p*) secretes a cyst wall round itself—the OÖCYST. Within this, the protoplasm contracts; and the nucleus then undergoes division into a variable number (4 in the diagram) of daughter nuclei (*q*). Around these nuclei the cytoplasm now segments, so as to form an equal number of rounded masses (*r*)—the SPOROBLASTS, or precursors of the spores. In this process a portion of the parent protoplasm is usually left over, forming an OÖCYSTIC RESIDUE (shown as a granular mass in *r*). Each sporoblast now secretes, in its turn, a cyst wall around itself—the SPOROCYST—and becomes a SPORE. Inside each spore, the nucleus divides (*r*), as a rule, forming a variable number (2 in the diagram) of daughter nuclei. The cytoplasm segments around these to form an equal number of uninucleate vermiform germs, or SPOROZOITES (*s*),—a small quantity of protoplasm being usually left over in the process to form a SPOROCYSTIC RESIDUE. The spores are now ripe. (Fig. *s* represents a ripe oöcyst, containing 4 spores (each inclosing 2 sporozoites) and an oöcystic residue.) Fertilization always takes place inside the host, but the formation of the

spores within the fertilized oöcyst (SPOROGONY) may take place outside. The oöcyst is usually very resistant, and serves to protect the spores during their development. The sporozoites, contained within the ripe spores, are the forms which are capable of infecting a fresh host. When living spores are ingested, and thus enter the gut, their walls burst and liberate the sporozoites (*i*). These then seek their suitable host-cells, which they invade (*f*) ; and thereupon they grow into the young parasites (*a*) with which our description began.

The development just described is seen, with various modifications, in all coccidia. It was first correctly made out by Schaudinn and Siedlecki in 1897, whose observations have since been abundantly confirmed. In the case of the intestinal coccidia of man, however, only certain stages of the sporogonic cycle are at present known : and though we must assume that the other stages are conformable with those already worked out in allied species, the schizogonic and sexual cycles are still unknown.

The CLASSIFICATION of the Coccidia is largely based upon the characters furnished by their oöcysts and spores. Of especial importance, from the systematic standpoint, are the size and shape of the spores, the number of them present within the oöcyst, and the number of sporozoites contained within each spore. The presence or absence of oöcystic and sporocystic residues is also of systematic importance.

The intestinal coccidia of man belong to two different genera—*Isospora* and *Eimeria*—which are readily distinguishable as follows:

Genus 1. ISOSPORA Aimé Schneider, 1881 (= *Diplospora* Labbé, 1893). Oöcyst containing two tetrazoic spores (*i.e.*, each inclosing four sporozoites).

Genus 2. EIMERIA Aimé Schneider, 1875 (= *Coccidium* Leuckart, 1879). Oöcyst containing four dizoic spores (*i.e.*, each inclosing two sporozoites).

In the human intestine there is but one species belonging to the first genus, but three distinct species in the second. Brief descriptions of all these will now be given.*

* The descriptions are, in the main, condensed from an earlier memoir by one of us (Dobell, 1919), in which a more detailed account of the Coccidia of man will be found.

(1) *ISOSPORA HOMINIS* (Rivolta) Dobell, 1919.

Chief synonyms ;

"Psorospermien" Virchow, 1860 ; Leuckart, 1863 ; Eimer, 1870.
Cytospermium hominis Rivolta, 1878.
Coccidium perforans (pro parte) Leuckart, 1879, et aliorum.
"Coccidies intestinales" Railliet & Lucet, 1890.
Coccidium bigeminum (Stiles) var. *hominis* Railliet & Lucet, 1891.
Coccidium hominis (Rivolta) Labbé, 1896.
Coccidium bigeminum (Stiles) Blanchard, 1896, et aliorum.
Eimeria stiedae (Lindemann) Lühe, 1906, et aliorum.
Isospora bigemina (Stiles) Doflein, 1911, et aliorum.
Isospora Wenyon, 1915, et aliorum.

This species was probably discovered in Berlin by a Swedish physician named Kjellberg, about the year 1860. His observations were briefly recorded by Virchow (1860). The parasites were seen in the villi of the small intestine. They appear to have been seen in a similar situation by Eimer (1870), and were first named, though not studied, by Rivolta (1878). The oöcysts were possibly first seen in the faeces, during life, by Railliet and Lucet (1890) ; but the first accurate account of them was given by Wenyon (1915, 1915*a*). Since then they have been studied by many workers.

The schizogonic and sexual cycles are completely unknown at present ; but presumably these stages occur in the small intestine, and resemble the corresponding stages in *Isospora rivoltae* Grassi (=*Coccidium bigeminum* Stiles)—a common parasite of the cat.

The OÖCYSTS, which are passed in human faeces in an incompletely developed condition, have the following characters. They have an elongate ovoid form (Pl. VI, figs. 97-102), the narrower end usually being drawn out into a sort of neck. They measure, as a rule, some 25-33 μ in length, by 12·5-16 μ in breadth at the widest part. More slender specimens are not uncommonly seen. The wall of the cyst (oöcyst proper) is thin, smooth, and colourless, and very resistant to most fixatives and other reagents. It consists of at least two layers—the innermost being thin and membranous, the outer hard and porcellaneous in appearance. At the narrower end an inconspicuous micro-

pyle—through which the microgamete entered—may sometimes be made out.

When discharged from the body in the stools, the oöcysts are usually unsegmented, their protoplasmic contents being contracted into a ball (Pl. VI, fig. 97), and filled with highly refractile granules of variable size. Among these the single nucleus is usually visible as a rather large clear area (fig. 97). Development takes place outside the body, and generally requires one or two days for its completion. The spherical mass of protoplasm first divides into two daughter masses (fig. 99)—the sporoblasts—its division being preceded by that of the nucleus (fig. 98). The two sporoblasts then rapidly become ovoid, and secrete cyst walls (sporocysts) around themselves, thus becoming converted into spores (fig. 100). The spores, as in other coccidia, have double walls—an inner and permanent wall (endospore), and an outer deciduous layer (epispore). They measure about 12-14 μ by 7-9 μ.

Further development takes place inside each sporocyst. The originally single nucleus divides, by two successive divisions, into four nuclei. Around each of these the cytoplasm collects to form the four worm-like or sausage-shaped sporozoites, each with a single nucleus at one of its ends. In this process of differentiation a large granular mass of protoplasm (sporocystic residue) is left over (fig. 100). This residue is at first very conspicuous in the spore, but later it disintegrates. There is no definite residue in the oöcyst itself in this species, though a few granules are sometimes visible—especially at the narrow end of the cyst (cf. fig. 99).

Degenerate oöcysts, which will not develop outside the body, are sometimes found in human faeces (figs. 101, 102): and an abnormal development, resulting in the formation of a single sporocyst containing eight sporozoites, has been observed (Wenyon and O'Connor, 1917).

When the spores are fully formed, no further development occurs outside the body. If the ripe cysts are swallowed by a human being they probably hatch in the small intestine, and liberate their contained sporozoites. These then become actively motile and invade the epithelium of the gut wall, where they grow and multiply. Such stages are, however, as noted already, still unknown in the present species: and they are postulated on the ground that a similar development occurs in related species found in other animals.

(2) *EIMERIA WENYONI* Dobell, 1919.

Synonym:

Eimeria (Coccidium) Wenyon, 1915.

This species was discovered and described by Wenyon in 1915.* It is a rare coccidium, and resembles *E. falciformis*, a common parasite of mice. The only stages hitherto seen are the fully developed oöcysts, discharged in human faeces. Their earlier stages of development, and the schizogonic and sexual stages of the parasite in the tissues of man, are still undiscovered; but it is probable that these earlier stages must be sought in the epithelium lining the small bowel.

The mature OÖCYST of *E. wenyoni* (Pl. VI, fig. 104) is approximately spherical, having a diameter of about 20 μ. Its outer surface is rough and rugose, its inner smooth and lined with a thin membrane. Contained within the oöcyst are four oval spores, measuring about 10μ by 7 μ. The sporocysts are superficially rough and somewhat irregular, owing, apparently, to the presence of persistent remnants of the episporal coats upon them. No oöcystic residue is present. Each spore contains two typical vermiform sporozoites, lying with their blunter nucleate extremities directed towards opposite poles of the spore. In addition to the sporozoites, each spore contains a sporocystic residue, in the form of one or two highly refractile rounded masses.

(3) *EIMERIA OXYSPORA* Dobell, 1919.

Synonym:

Eimeria oxyphila Mesnil, 1919.†

This species of *Eimeria* was described by Dobell (1919), and is, like the preceding, apparently a rare organism. Up to the present the fully developed oöcysts are the only stages which have been seen. They differ greatly from those of *E. wenyoni*.

The fully formed OÖCYSTS (Pl. VI, fig. 103), as passed in the stools, are large spherical structures measuring about 36μ in diameter. Each contains four long, whetstone-shaped spores and a small oöcystic residue in the form of a few bright granules. The wall of the cyst (oöcyst

* See Wenyon (1915*b*).

† The employment of this name by Mesnil appears to be inadvertent, and merely the result of a misprint.

proper) is slightly yellowish, and composed of at least two layers—the inner thick and uniform, and the outer, which appears composite incrusted with foreign particles from the faeces.

The four spores are provided with tough walls (sporocysts), and appear slightly rough externally—especially at their ends—owing to the presence of persistent remains of the epispores. The spores are equally sharply pointed at both ends, and measure 30-32 μ in length by 7·5 μ in breadth at the middle. Each contains two very long sporozoites and a sporocystic residue in the form of a number of small highly refractile granules. The sporozoites are blunt at one end, pointed at the other, and lie with their blunt ends directed towards opposite poles. Each has a large oval nucleus at the blunt end, situated subterminally. Between the nucleus and the blunt extremity (probably the posterior end of the sporozoite), there are always two or three minute fusiform bodies, of unknown nature. They are very brightly refractile, and resemble crystals. At the other side of the nucleus—that is, in the cytoplasm between it and the pointed end—a few rather feebly refractile granules can be made out.

The other stages of this parasite are unknown, but probably occur in the epithelial lining of the small intestine.

(4) *Eimeria snijdersi* Dobell, 1921.

This species was recently discovered by Snijders (1921) in Sumatra. It somewhat resembles the preceding form, and, like it, is known only from the fully developed oöcysts found in the stools.

The oöcysts (Pl. VI, fig. 105) are very large—even larger than those of *E. oxyspora*. They are spherical, and measure 40μ to 48μ in diameter—the average being about 45 μ. Their walls consist of at least two layers, and inclose the four dizoic spores typical of the genus, and a small oöcystic residue in the form of a few scattered granules.

The spores are like those of *E. oxyspora*, but are relatively shorter, and spindle-shaped (fig. 105). They have the usual double sporocysts (permanent endosporal and deciduous episporal coats), and contain two long vermiform sporozoites and a small sporocystic residue in the form of one or two refractile globules. The length of the spores is about 22μ to 24μ, their breadth 7·5μ. No "crystalline" bodies, like those of *E. oxyspora*, have been observed at the blunter ends of the sporozoites.

E. snijdersi thus differs from *E. oxyspora* in having larger oöcysts and shorter and relatively plumper spores. Its habitat in the body is still unknown ; but, like the other species of *Eimeria* in man, it probably lives in the epithelium of the small intestine. Snijders (1921) observed a few unsegmented—probably degenerate—oöcysts in the faeces of the only infected individual whom he studied ; but apart from these, no other stages in the life history have yet been seen.

Key to the Genera and Species.

We give below a simple key for the determination of the genera and species of coccidia hitherto found in human faeces—a key based entirely upon the characters supplied by the oöcysts and spores, since the other stages are still insufficiently known.

1. (*a*) Ripe oöcyst elongate, with 2 tetrazoic spores — Genus *Isospora* 2.
   (*b*) Ripe oöcyst spherical, with 4 dizoic spores ... — Genus *Eimeria* 3.
2. Spores oval, 12-14 μ × 7-9 μ ... ... *I. hominis.*
3. (*a*) Oöcyst ca. 20 μ ; spores oval, ca. 10 μ × 7 μ ...*E. wenyoni.*
   (*b*) Oöcyst ca. 36 μ ; spores whetstone-shaped, ca. 31 μ × 7·5 μ ... ...*E. oxyspora.*
   (*c*) Oöcyst ca. 45 μ ; spores spindle-shaped, ca. 23 μ × 7·5 μ ... ...*E. snijdersi.*

Intestinal Coccidiosis.

Coccidiosis is the general name given to the condition of being infected with coccidia, but we are here concerned only with intestinal coccidiosis in man—a subject about which very little is known.

The commonest intestinal site of infection with coccidia—speaking generally—is the small intestine : but species of coccidia which inhabit the stomach (*e.g.*, *Cryptosporidium* in the mouse) and the large gut (*e.g.*, *Eimeria zürni* in cattle) are known. From the fact that Kjellberg* and Eimer (1870) appear to have observed coccidia in the epithelium of the small intestine of man, and since oöcysts have since been found in human faeces, it thus seems probable that the small bowel is the site of infection selected by at least one of the coccidial parasites of man.

Since the Coccidia are always tissue-parasites, they must always

* See Virchow (1860).

produce a more or less pathological condition in their host. Nevertheless, no clinically recognizable disease due to their presence has yet been observed in man. Even in those cases in which, from the number of oöcysts passed in the stools, a heavy infection appears to have been present, no definite symptoms referable to the infection have been elicited. This is in agreement with the observations made upon coccidiosis in other animals. The Coccidia parasitize both vertebrates and invertebrates, and frequently occur in their hosts' tissues in immense numbers: but in spite of this, their hosts often appear to be unaffected, in genera health, by their presence.

In agreement with this apparent lack of pathogenicity, the lesions due to coccidial invasion show, in most animals, surprisingly little tissue reaction. At the site of infection there is often little to be seen but cellular destruction, the surrounding tissues appearing perfectly healthy. Sometimes, however, the parasites apparently cause considerable hypertrophy of the neighbouring tissues—a condition usually seen in the well-known hepatic coccidiosis of the rabbit. *Eimeria stiedae*, the parasite here implicated, invades the epithelium of the bile ducts, which frequently undergo, in consequence, a prodigious proliferation. Heavy infection, moreover, may lead to serious consequences, as is well seen in the case of *E. stiedae* and *E. zürni*. The former may cause a fatal disease in the rabbit, and the latter causes sometimes a severe and even fatal form of dysentery in cattle. Apart from such inferences as can be drawn from similar examples of coccidiosis in animals, nothing definite can yet be said about the pathology, morbid anatomy, pathogenesis, or symptomatology, of intestinal coccidiosis in man.

It should be noted that a condition of hepatic coccidiosis has been described in man. It appears to be due to a species of *Eimeria* which has not yet been properly investigated. The parasite was discovered in France by Gubler (1858), and has since been observed, apparently, by several other workers.* The oöcysts of this species are oval structures, measuring some 20 μ in length: and it must be presumed that they are passed out in the faeces of infected individuals. Further information about this coccidium is much needed. It has usually been supposed that it is identical with *E. stiedae* of the rabbit, but this is almost certainly incorrect.

* For a full account of this organism—so far as it is known—see Dobell (1919) p. 190.

All the intestinal coccidia of man are still very imperfectly known. It was thought until recently that they were of the same species as those occurring in rabbits, cats, and dogs: but this is not correct. The parasites of man appear to be peculiar to man,* and careful attempts which have been made to transmit them to other animals (by Wenyon and O'Connor) have all been completely negative. It should also be noted here that many of the earlier cases of "coccidiosis" described in man are now known to have been based upon mistakes of observation and interpretation.

Intestinal coccidiosis is a rare condition in man. By far the commonest species hitherto found is *Isospora hominis*, with which over 70 cases of infection have now been recorded (Wenyon, 1915, 1916; Woodcock and Penfold, 1916; Roche, 1917; Cragg, 1917; Brumpt, 1918; and others). All the infections were, apparently, observed in persons who had been in Egypt, Gallipoli, Greece, Mesopotamia, and neighbouring countries. It is possible, therefore, that the infection is endemic in these areas.† *E. wenyoni* was found by Wenyon (1915*b*) in a single patient who had been in Gallipoli. Three further cases were observed by Roche (1917) in Salonika, and Chatton (1918*a*) and Brumpt (1918) appear to have seen a few more in Tunis and France respectively. Nothing else is known about the distribution or incidence of this parasite.

E. oxyspora and *E. snijdersi* have been seen but once each—both of them in patients suffering from chronic amoebic dysentery acquired in the tropics. The patient with *E. oxyspora* came from Ceylon, the one with *E. snijdersi* was studied in Sumatra. It is still too early to draw conclusions regarding the distribution of these parasites, therefore, though there is some evidence to show that all the species of *Eimeria* found in man have a tropical or a subtropical habitat.

In conclusion, it must be noted that coccidiosis in man appears to

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* Reichenow (1920), in a work just published, appears to dissent from this view, on the grounds that the coccidia hitherto found in man may ultimately be found to parasitize other hosts. But this is an objection which may be urged against any parasite whatsoever which has ever been described from any host, and one which can never be disposed of until all the parasites of all animals are fully known. At present the coccidia found in man have been shown to occur in man only: they have been found in no other host. Until they have been shown to occur elsewhere, we see no impropriety in saying that they are "peculiar to man." This is a true statement of fact as at present ascertained. (C. D.)

† Noc (1920) has recently described a case of *Isospora* infection found in Senegal in a French soldier who had never been in Macedonia or the Dardanelles.

be a transient condition. In most of the cases hitherto observed the oöcysts have been found in the stools on very few occasions, and have disappeared completely when the infected individuals were kept under observation. All the species of *Eimeria* were seen to behave in this way. More persistent infections have been observed, however, in the case of *Isospora* (Roche, 1917 ; O'Connor, 1919). There is some reason to suppose that many coccidial infections are transitory, and tend to die out of their hosts with time. There is nothing to show that the schizogonic cycle can be repeated indefinitely ; and the occurrence of oöcysts in the stools may mark the completion of development and the final exit of the parasite. Heavy and persistent infections, often seen in animals, are probably a result of frequent re-infection ; and the transitory character of human infections may be due to the circumstance that such re-infection has generally been prevented.

CHAPTER VI.

THE INTESTINAL CILIATES OF MAN. BALANTIDIOSIS.

THE protozoal Phylum known as the CILIOPHORA contains an immense number of organisms. It is represented in the human bowel, however, by very few species—at least one, and possibly as many as three or four. All these belong to the Class CILIATA (or Infusoria, *sensu stricto*) and its Order HETEROTRICHA.

A typical ciliate—such as the familiar *Paramecium* of ponds—is a moderately large protozoon, more or less bilaterally symmetrical, and covered more or less completely with a coat of hair-like cilia, which serve for locomotion. It has a permanent mouth and other organs—such as nuclei, contractile vacuoles, etc.—and shows a considerable degree of structural complexity. It multiplies by transverse fission into two. The typical ciliate is hermaphrodite, and has a remarkable and peculiarly complicated sexual process called conjugation. Encystation occurs at some time in the life-cycle of most species.

One of the most characteristic features of the Ciliata, besides their cilia, is their nuclear apparatus. This consists of two nuclei, or two sets of nuclei, known as meganucleus (or macronucleus) and micronucleus. The former are to be regarded as somatic nuclei, the latter as germinal nuclei—respectively comparable with the nuclei of the body-cells and germ-cells of a metazoon.

The ciliates found in the human bowel belong to the less specialized forms, and have a comparatively simple structure and life-history. They belong to the genera *Balantidium* Claparède & Lachmann, 1858, and *Nyctotherus* Leidy, 1849. The species will now be described in detail.* There are three which appear to be valid, and several doubtful forms which require further investigation.

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* A key to the genera and species will be found on p. 118 *infra*. From the systematic standpoint, the genera—which have been long established, and are well known—require no special discussion here.

(1) *BALANTIDIUM COLI* (Malmsten) Stein, 1862.

Chief synonyms:

Paramaecium ? coli Malmsten, 1857.

Plagiotoma coli (Malmsten) Claparède & Lachmann, 1858.

Leucophrys coli (Malmsten) Stein, 1860.

Holophrya coli (Malmsten) Leuckart, 1863.

This, the commonest of the intestinal ciliates found in man, was discovered* in 1856 by Malmsten, a Swedish physician, in Stockholm. He described and named† it in the following year—his account of the parasite being accompanied by admirable figures executed by the zoologist Lovén. Malmsten found the organism in the stools of two patients suffering from dysentery: but his discovery was extended soon afterwards by Leuckart and Stein, who found that the organism occurs very frequently in pigs. Many analogous observations have been since recorded by later workers, and the parasite has been many times redescribed. In recent years it has also been found in monkeys.

Balantidium coli is the largest protozoon encountered in the human intestine. It is roughly oval in shape, and in length usually measures—in our experience—from about 50μ to 70μ, with a breadth, at the widest part, of some 40μ to 60μ. Different authors have given various dimensions for the forms which they have studied; and these range from as little as 25μ, as a minimal length, up to over 200μ as a maximum. Many observers describe specimens attaining a length of 100μ or even more. In the free-living ciliates—such as *Paramecium*—it is now well known, as a result of the work of Jennings and others, that many species are composed of a number of distinct races distinguishable by their average sizes.‡ The species *Paramecium candatum*, for example, is divisible into at least half-a-dozen such races, whose mean lengths range from about 230μ to about 175μ. It seems highly probable that further investigation will show that similar races, differing in their dimensions, exist in *Balantidium*.

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* It is frequently stated—owing to a series of mistakes—that this organism was discovered by Leeuwenhoek. See Dobell (1920) for the history of this matter.

† Malmsten (1857) referred the parasite to the genus *Paramecium* with some doubt. He says: "Da diese Darminfusorien sich am meisten den Paramaecien zu nähern scheinen, so könnte man die Art einstweilen *Paramaecium? coli* nennen." *Op. cit.*, p. 305.

‡ Full references to the literature of this subject will be found in an earlier work by one of us, Dobell (1914).

The general form and structure of *B. coli* is shown in fig. 109 (Pl. VII), and somewhat diagrammatically in fig. 106 on the same Plate. The latter figure depicts an individual viewed from the left side. The animal is slightly asymmetrical, and more pointed at the anterior than at the posterior end. Situated subterminally at the anterior end is a small triangular area leading into a funnel-like pit—the mouth (*mo.*). The surface of the body on which this is placed is called ventral, the one opposite to it dorsal. The dorsal surface is rather more convex than the ventral, and frequently appears to bulge somewhat in consequence. The asymmetry is especially conspicuous when the animal rotates in the process of swimming. As a rule it retains its shape unaltered; but it is not rigid, and is often seen to be bent or distorted by the pressure of the surrounding bodies among which it moves in the faeces. *Balantidium* is generally described as being "slightly metabolic," but its changes in shape appear to be produced passively.

The whole body is invested with a coat of very fine cilia, arising in parallel longitudinal rows from minute basal granules. The rows of cilia give the animal the appearance of being striated. The main part of the body consists of granular endoplasm, in which the internal organs are situated. This is surrounded by a thin layer of ectoplasm, of a clear, alveolar structure, and the whole body is invested externally by a very thin and delicate cuticle, through which the cilia emerge. The cilia within the mouth are longer than those on the general body surface. The mouth itself is not quite symmetrical; and it contains, according to some observers, a delicate undulating membrane. The triangular ciliary field in front of the mouth has a somewhat specialized structure, as in other Heterotricha, and is termed the peristome. It appears to be a region specialized for "tasting" and capturing food. The mouth itself leads into a gullet, which is very short, its narrow internal opening ending abruptly in the endoplasm.

The single large meganucleus (*N*) lies near the middle of the body, deeply imbedded in the endoplasm. It is kidney-shaped or bean-shaped; but as it lies transversely, it often appears oval in sideview. It contains densely packed chromatin granules, and a few larger nucleoli, and is bounded by a membrane. The micronucleus (*n*) is very small, and spherical. It usually lies closely applied to the meganucleus, in the depression or bay on its ventral surface.

Two rhythmically contractile vacuoles are present in this species.

One lies more anteriorly in the mid-dorsal region (*c. v.* 1), the other dorsally at the hind end (*c. v.* 2). In the living animal they frequently appear to be connected by a system of lacunae, or ducts with accessory vacuolar dilatations. They pulsate slowly, and are often difficult to make out—especially the anterior one. At the extreme hind end of the body there is a minute obliquely placed duct-like structure, permanently opening to the exterior. It is usually termed the "anus"; but to judge from its relation to the posterior contractile vacuole—into which it sometimes appears to open—it is probably the duct of the vacuolar system.*

In addition to the foregoing structures, the endoplasm contains more or less numerous food vacuoles (*f. v.*), containing ingested matter. Food particles are taken in at the mouth, pass through it into the endoplasm, and there become surrounded with a drop of liquid to form a food vacuole, in which digestion takes place. These vacuoles, which thus function as stomachs, circulate in the endoplasm during the process of digestion. When the contained food has been digested, the insoluble faecal residue is cast out of the body. †

B. coli ingests all manner of faecal débris in its host's intestine. It also eats red blood-corpuscles, when these are available, leucocytes, and tissue fragments. Starch grains are often seen in the vacuoles, and Glaessner (1908) has found that the organism contains a diastatic ferment. He also extracted a haemolysin, but no proteolytic ferment.

This ciliate lives in the more fluid part of the contents of the large intestine—especially in the caecum. It has also been found in the appendix. At times it is found deeply imbedded in the tissues, which it is apparently able to attack and destroy (*vide infra*).

Like most other ciliates, *B. coli* multiplies by TRANSVERSE FISSION into two. All the stages of division have not been carefully studied, but the chief stages have been seen by various observers, and there can be little doubt that its division is like that of other species of the genus. The micronucleus first divides by mitosis: then the meganucleus is constricted into two (amitosis): finally the cytoplasm constricts transversely, and two daughter individuals are thus formed. The posterior individual forms a new mouth at its anterior end, and more or less

* These remarks are based upon observations on *B. coli* from pigs. (C. D.)

† Through the "anus," according to some workers. We have not been able to confirm this observation.

extensive reorganization and reconstruction of the ciliary coat and other parts occurs in both individuals.

A remarkable process of "budding" was described by the earlier Russian workers, and a process of "sporulation" has been more recently described by Walker (1909), but these accounts seem open to question. No such processes are known to occur in related ciliates.

CONJUGATION has been described by some of the early observers and more recently by Brumpt (1909, 1913) in this species; but the phenomenon has not been observed by most other workers, and requires further investigation.

B. coli encysts in the intestine, and its CYSTS pass out, like those of other protozoa, with the faeces of its host. The animal rounds itself off, secretes a cyst wall, and after revolving actively inside it for some time, comes to rest. Its ciliary covering then degenerates more or less completely. Food bodies are digested or eliminated before encystation, and the most conspicuous structure in the cyst is the meganucleus (Pl. VII, fig. 110). Irregular refractile bodies (sometimes said to be fat) are often present in newly formed cysts, and the posterior contractile vacuole persists for a considerable time, pulsating rhythmically. The cyst wall is colourless or slightly yellowish, fairly thick, and very tough. It consists of two distinct layers—outer and inner, the former being the thicker. The cysts are round, or slightly ovoid, and commonly measure 50 μ to 60 μ in diameter. They are thus the largest protozoal cysts encountered in human faeces.

As a rule the cysts contain a single individual, but specimens containing two individuals have been described. It is not clear whether these are formed as a result of the division of the originally single organism within the cyst—as seems most probable—or from the encystation of two individuals together. According to Brumpt (1909) two individuals may associate and form a common cyst, in which, later, they fuse.

Infection is acquired by swallowing the cysts, which probably hatch in the small intestine: but the details of the process have still to be investigated. The cysts are able to live for a considerable time—at least several weeks—in faeces, in which they remain apparently unchanged if prevented from drying.

(2) *BALANTIDIUM MINUTUM* Schaudinn, 1899.

This small and apparently rare ciliate was described some years ago by Schaudinn (see Jakoby and Schaudinn, 1899). It has the following structure.

The body (Pl. VII, fig. 107) is oval or somewhat piriform—its breadth being about two-thirds of its length. The dimensions vary from 20-30 μ by 14-20 μ.* The anterior end is somewhat pointed, but appears slightly truncated and bent to one side: the posterior end is plump and rounded. The cilia on the body are very long and fine. The buccal apparatus is more strongly developed than in *B. coli*. It consists of a relatively long peristomial groove, extending from the anterior extremity to the equator—or even further backwards—and there ending in a short gullet which sinks into the endoplasm. The mouth-parts are furnished with long vibratile cilia.

There is only one contractile vacuole, which lies dorsally, on the left side, at the hind end of the body.

The meganucleus is spherical, measuring 6-7μ in diameter, and centrally placed. It is surrounded by a delicate membrane, and contains irregular chromatin granules arranged on a linin network. The micronucleus is a minute sphere, about 1 μ in diameter, and usually lies in front of the meganucleus.

Multiplication is effected in the typical manner by transverse division. Conjugation was not observed: but encystation is said to occur in the usual way—the cysts being usually oval, however, and not spherical (dimensions not stated).

Schaudinn studied these ciliates in only two infections—one found by Jakoby, the other by Schulz,† in Berlin. Both patients suffered from diarrhoea, and their stools contained the organisms in immense numbers. No evidence of the pathogenicity of this species was obtained, however; and Schaudinn was of the opinion that it is probably harmless.

Brooks (1903) states that "Dr. Russell" found *B. minutum* in the stools of soldiers in Porto Rico, but we have been unable to find any further account of these cases.

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* These are the dimensions recorded by Schaudinn: but it should be noted that the measurements stated in the text do not agree with those of his figures. I assume that his description is correct, and that the magnification of his figures is wrongly stated. The same remarks also apply to his account of *Nyctotherus faba*. (C. D.)

† This case is very doubtful; see p. 115, footnote, *infra*.

Recently Sangiorgi and Ugdulena (1917) have found a ciliate which they appear to regard as a variety of *B. minutum*, but which they propose to call "*Balantidium minutum*, sp. *Italicum*" (meaning "var. *italicum*" ?). They found the organism in the stools of a soldier, and were able to cultivate it easily in peptone-water.* Its size varied from 28·8 μ to 36·8 μ, by 11·2 μ to 25·6 μ, and it is stated that in cultures it formed cysts measuring 12·8 μ by 11·2 μ. The meganucleus is said to have an antero-lateral position. From this description it thus appears doubtful whether the organism was really a *Balantidium* at all; and it seems more probable that it was a free-living ciliate which had accidentally gained access to the stools. A rough figure of the organism, which appears to confirm this interpretation, has more recently been published by Sangiorgi (1919).

Pinto (1919*a*) has lately recorded the finding of *B. minutum* in Brazil (State of Paraná); but he gives no description of his organism so named—merely recording that it was present in 5 out of 3,917 samples of faeces examined at various places.

It will be seen that our knowledge of *B. minutum* is still very defective. The only certain case in which it has yet been found appears to be that studied by Jakoby and Schaudinn.

(3) *NYCTOTHERUS FABA* Schaudinn, 1899.

A single case of infection with this organism has been described by Schaudinn (see Jakoby and Schaudinn, 1899). The patient in whose stools the ciliates were found suffered from diarrhoea, and was also infected with *Balantidium minutum*.

The organism (Pl. VII, fig. 108) is bean-shaped, and somewhat flattened dorso-ventrally. The peristomial region extends from the anterior end backwards to the middle of the body, where it terminates in a short, oblique gullet, which enters the protoplasm as a narrow tube. The body is clothed with short and fine cilia, whilst the peristome is furnished with longer and stronger ones. The length of the body is 26-28 μ, its breadth 16-18 μ.† It is thus the smallest species of the genus yet described.

* See also, in this connexion, Sangiorgi (1918*b*).

† See the remarks on Schaudinn's measurements of the preceding species—p. 111, footnote, *supra*.

There is a single large contractile vacuole, situated at the posterior end, and discharging its contents through a tubular duct or "anus" like that of *Balantidium coli*.

The meganucleus is a centrally placed sphere, measuring 6-7 μ in diameter. Its structure is peculiar, in that the chromatin granules are massed into four or five large blocks. The micronucleus is spherical or comma-shaped, measures some 1-1·5 μ in diameter, and is closely appressed to the meganucleus.

Neither division nor conjugation was seen in this species. It is stated to form oval cysts (dimensions not recorded), distinguishable from those of *B. minutum* by the characteristic structure of the meganucleus.

No evidence was elicited to indicate that this organism is pathogenic, and Schaudinn believed it to be harmless.*

A ciliate believed to belong to this species has been recently described, from the stools of a soldier in Italy, by Sangiorgi and Ugdulena (1917). They were able to cultivate it, and give its length as 20·8 μ to 57·6 μ. It was considerably larger, therefore, than Schaudinn's specimens. From the rest of their description, also, it appears doubtful whether this ciliate was really *N. faba*. It is said, however, to have possessed a similar meganucleus, and to have had a single posterior contractile vacuole: and it is described as forming cysts measuring 11·2 μ in diameter. The identity of this ciliate appears to be still questionable.

Pinto (1919*a*) states that he has found one case of *N. faba* infection in Brazil, but he gives no details.

It is thus clear that further information about *Nyctotherus faba* is much to be desired. Schaudinn's case appears to be the only certain one on record.†

Doubtful Ciliates.

Under this heading we must now briefly notice several ciliates which have been found, at various times, in human faeces, but which are of doubtful systematic status and questionable relation to man.

Most of these doubtful forms have been seen but once, and not one of them has yet been studied or described by a recognized authority

* It may be remarked that no pathogenic species of this genus is known. Other species occur in frogs, cockroaches, etc.

† Cf. p. 115, footnote, *infra*.

upon the Ciliata. Consequently—since the identification of genera and species among ciliates is by no means easy for the inexperienced—the opinions recorded by the describers are not always such as a protozoological systematist can unreservedly accept.

Moreover, it seems highly probable that most, if not all, of these doubtful forms were, in reality, free-living species which had accidentally contaminated the material examined. In most cases there is insufficient evidence to prove that the "parasites" discovered were true entozoic organisms: and the circumstance that they have sometimes been identified—more or less accurately—as well known free-living species, also points to the same conclusion. It must be remembered that free-living ciliates may occasionally be found in the water or saline solution used in diluting faeces for examination,* and the contamination of stools may also occur in other ways. It should also be remembered that no free-living species belonging to entozoic genera—such as *Balantidium*—are known, though many have been described in error.

The first case which we must note is that of Guiart (1903), who described a ciliate from the diarrhoeic faeces of a Frenchwoman. He believed it to have been present in the intestine, but was able to cultivate it by adding water to the faeces. The organism measured 35-55 μ by 25-35 μ, and was identified as "*Chilodon dentatus* Dujardin." Later, Manson and Sambon (1909) reported the finding of a similar organism in the stools of "a person apparently healthy who had just returned from Northern Rhodesia." It was identified as "*Chilodon uncinatus* Blochmann," and its dimensions were given as 36-44 μ by 20-30 μ. From the descriptions it seems clear that all these authors were really dealing with *Chilodon:* but it is difficult to believe that in these cases the "infections" were not really due to contamination, in some way, of the stools examined. *Chilodon* (several species) is one of the commonest of free-living ciliates, occurring in water and infusions everywhere; but no entozoic species are known. Further evidence is needed, therefore, to prove that any species† can occur in man. It should be noted that Manson and Sambon regarded their case as one of "pseudo-parasitism."

* See p. 139, *infra.*

† There are, properly speaking, no such species as "*C. dentatus* Dujardin" and "*C. uncinatus* Blochmann." The genus *Chilodon* was founded by Ehrenberg in 1833, and *C. uncinatus* was one of his species. *C. dentatus* was a combination of names introduced by de Fromentel in 1874—probably for the same species, and is therefore merely a synonym. It appears probable that both Guiart, and Manson and Sambon, observed this species—*i.e.*, *C. uncinatus* Ehrbg., one of the commonest species of the genus, occurring in water almost everywhere. *Chilodon* is also known, it may be added, as an external parasite of freshwater fish (cf. André, 1912). (C. D.)

The finding of *Colpoda cucullus* in human faeces has been reported by Schulz (1899). This observation—if correct—must assuredly have been due to contamination of the material examined: for this organism, which is extremely common in infusions of many sorts, never occurs, so far as is known, inside other animals—being an exclusively free-living form.*

Difficulties of a different sort are presented by a remarkable organism described under the name of "*Nyctotherus africanus*" by Castellani (1905). He found it in "a Baganda native affected with sleeping sickness." According to the description it is roughly hour-glass shaped, with a meganucleus and micronucleus, a contractile vacuole, and a "peristome" which is said to be "on the posterior zone." The animal is stated to measure 40-50 μ by 35-40 μ, and to be covered with extremely fine cilia. From the description and figures of this organism we can only say with certainty that it is not a *Nyctotherus*.† We cannot determine its true systematic position, since we know of no other ciliate displaying similar morphological peculiarities.

In the following year, Krause (1906) described a ciliate which he found, in Germany, in the faeces of a young woman with typhoid fever. He was able to preserve it alive outside the body for 5 weeks, in an

* The statements of Schaudinn and Schulz regarding this case are not easy to reconcile, and raise a number of doubts in my mind. Schaudinn (Jakoby and Schaudinn, 1899) says that a second case of infection with *Balantidium minutum*—in addition to Jakoby's—was shown him by Schulz, who was then about to describe it. Schaudinn appeared to be in no doubt regarding the identity of the organism. In his own paper, however, Schulz (1899) states that he found a ciliate which at first he thought to be "Balantidium Protozoon" (*sic*), but which was identified *by Schaudinn himself* as *Colpoda cucullus*. He adds that Schaudinn told him that this organism had been found living parasitically only once previously—by Küchenmeister, in a horse: but he makes no mention of *B. minutum*. (It is true that these authors misspell one another's names—Schaudinn referring to "Dr. Schultz," and Schulz returning the compliment by alluding to "Dr. Schandinn": but their identity can hardly be in doubt.) Prowazek (1914) gets over the difficulty by simply stating that Schulz described a case of *B. minutum* infection, but called the organism *Colpoda cucullus*. It is very difficult, however, to reconcile the statements of Schaudinn and Prowazek with those of Schulz; and it is almost inconceivable that any protozoologist could confuse two such very different organisms as *B. minutum* and *C. cucullus*. It seems to me remarkable, further, that although Schaudinn described *B. minutum* and *N. faba* as long ago as 1899—since when hundreds of thousands of human stools have been examined—no real confirmation of his findings has been forthcoming. If Schulz's case is eliminated as too doubtful to be accepted, then these two organisms have each been found once only, and then both in the same patient. In view of this singular state of affairs, I add Schaudinn's two ciliates to the intestinal protozoa of man with much misgiving. (C. D.)

† The only real reason which Castellani appears to have had for placing this animal in the genus *Nyctotherus*, seems to be the peculiar structure of its meganucleus, which is described as similar to that of *N. faba*. But this organism itself—whatever it may have been—has a meganucleus quite unlike that of any other *Nyctotherus* which (so far as I know) has ever been described. (C. D.)

alkaline medium. It is described as an oval organism, 90-400 μ long by 60-250 μ in breadth.* There are said to be two contractile vacuoles, but the cilia are not arranged in rows. Owing to the methods of fixation and staining employed, it is impossible to attach much importance to the other cytological characters noted. The figures depict, apparently, distorted specimens with broken-down nuclei. Cysts were observed, and are figured as roughly spherical. Krause proposed† to call the ciliate "*Balantidium coli giganteum.*" According to Doflein (1916) and others, this organism should be placed in the genus *Nyctotherus*, and therefore called *N. giganteus.* We cannot concur in this view, for we are unable to determine the systematic position of the ciliate from the imperfect description and obviously faulty figures hitherto published. It is possible—if the measurements are correct—that it was a strain of *Balantidium coli* of unusually large size, but we cannot advance this hypothesis with any confidence. We believe, in any case, that there is no evidence that it was a *Nyctotherus.*

Martini (1910) observed a little oval ciliate, with a large peristome and a long caudal filament, in the stools of three patients suffering from dysentery in Tsingtau. The organism measured 30-43 μ by 11-15 μ, and it was found that it would live for some weeks, and even multiply, in saline solution containing faeces and kept at room temperature. Martini proposed—on grounds which seem inadequate, to say the least—to call the organism "*Uronema caudatum,*" and to add it to the list of human "parasites" capable of "causing" dysentery. Fischer (1914) believes that he has been able to confirm these observations at Shanghai. From the published description and figures, however, we believe this to have been another free-living ciliate, and not one which occurs in the human intestine.‡

Castellani (1914, 1914*a*) has described an organism, found in human stools, under the name of "*Entoplasma.*" From the description we cannot identify it: but from a preparation of the organism which the

* Krause (1906), p. 446. On p. 451 the breadth is given as 60-150 μ.

† This name is proposed on p. 452, *op. cit.* In the title the animal is called "Balantidium giganteum."

‡ It seems clear from Martini's account that his organism was not a *Uronema.* The genus *Uronema* was founded by Dujardin (1841), the type species being *U. marinum* Duj. Martini's form appears to be a *Cyclidium*—as now defined. Species of this genus are very common in water, and infusions of all sorts, all the world over. These genera, and those allied to them, have been accurately studied and defined by Bütschli, Schewiakoff, Roux, and others—in works well known to all protozoologists. (C. D.)

author kindly showed to one of us (C.D.), we have formed the opinion that it was probably a *Balantidium*, or some other ciliate, which had been deformed by drying and the method of preparation adopted.* Brug (1918*a*), however, has suggested that "*Entoplasma*" is a dried and deformed *Chilomastix*, but its large size disproves this interpretation.

The "*Balantidium*" obtained in cultures from the spleen by Marshall (1911), and that found in the blood and in blood-cultures by Hinkelmann (1919) must also be mentioned here, as they must at present be included among the doubtful ciliates. They will be noticed in more detail later (*vide* p. 121 *infra*).

Barlow (1915) believes that a "*Balantidium*" which he has found in human stools in Spanish Honduras is a distinct variety of *B. coli*, and proposes for it the name "*Balantidium coli*, variety *Hondurense*." The organism is said to be uncommon, and to measure 100-175 μ by 70-100 μ. It has a smaller mouth than the type, and shows no ciliary striation. But its most remarkable peculiarities are that its "nucleus" is inconspicuous and spherical (only 10-14 μ in diameter), whilst "what appeared to be a kineto-nucleus could occasionally be made out." "Only rarely" is a "vacuole" present, and then it is not contractile: but to make up for this, the organism possesses an "anal orifice" which is "very contractile." It seems clear that this animal can hardly be placed in the genus *Balantidium*, and it certainly cannot be regarded as a variety of *B. coli*. It may be suggested that the author's observations were inexact, and that he was possibly dealing with a free-living ciliate belonging to a different genus.

This interpretation undoubtedly applies to the ciliate recently described under the name of "*Balantidium coli* sp. *Albanense*" by Sangiorgi (1919). From the rude figure illustrating his description, and from the circumstance that the organism was found in wells in Valona (Albania), there can be no doubt that it was a free-living species (unidentifiable from the incomplete account given), and not a variety or species of *Balantidium*.

Key to the Genera and Species.

We give below a simple key to the genera and species of ciliates which occur in the human intestine. We include only those species

* We understand that this is also the opinion of Dr. C. M. Wenyon, who first suggested this possibility to us.

which have been sufficiently studied for it to be possible to identify them with certainty—all the doubtful forms just mentioned being left out of account. The characters utilized for the purpose of determination are those of the full-grown active organisms.

1. (*a*)	Body oval	Genus *Balantidium* 2.
(*b*)	Body bean-shaped	Genus *Nyctotherus* 3.
2. (*a*)	Peristome very short, subterminal; meganucleus kidney-shaped; 2 dorsal contractile vacuoles. Length 50 μ or more ...	*B. coli.*
(*b*)	Peristome long, extending to middle of body; meganucleus spherical; 1 posterior contractile vacuole. Length 32 μ or less	*B. minutum.*
3.	Peristome extending to middle of body; meganucleus spherical; 1 posterior contractile vacuole. Length 28 μ or less	*N. faba.*

Balantidiosis.

Infection with *Balantidium coli* is termed Balantidiosis (or Balantidiasis). The term is applicable, of course, to infection with any species of the genus. The other ciliates of man are both rare and—so far as is known—harmless, so that no special term has been applied to the conditions with which they are associated. On the other hand, *B. coli* is, at times, a pathogenic parasite, and produces a definite disease with characteristic symptoms and lesions.

Pathogenesis, Aetiology, etc.—It appears probable that *Balantidium coli* is a natural parasite of the pig, to which it appears to be usually harmless. It may also be a natural inhabitant of monkeys, for it has been found in these animals in several parts of the world. Man appears to be an accidental host, and to acquire his infection, as a rule, from the pig.

When a man becomes infected with the parasite he often displays no symptoms, and becomes a carrier—like a carrier of *E. histolytica*. The relation of parasite and host in such circumstances is not yet fully understood: and it is not certain whether the ciliate attacks the tissues

of the gut, or lives as a harmless commensal upon its contents—as other species of the genus commonly do in other hosts. In man, however, *B. coli* is at times a definite tissue-parasite. It attacks and invades the mucous and submucous layers—sometimes even the muscular layers—of the large intestine, and produces an ulceration closely resembling that seen in *E. histolytica* infection. The result is a condition of colitis, with symptoms of diarrhoea or, in severe cases, dysentery (Balantidial Dysentery, or Ciliate Dysentery). Secondary infection of other organs, such as is sometimes seen in amoebiasis, probably does not occur.

This disease is most prevalent among people who tend pigs or handle their carcases—swineherds, farm labourers, slaughterers, sausage-makers, etc. Strong (1904), in a review of all the cases of human balantidiosis then known, found definite evidence of association with pigs in 25 per cent. of the patients. Since then, several striking cases—such as that of Young and Walker (1918)—showing the aetiological relation of the pig to human infection, have been reported.

Incidence and Distribution.—Balantidial infection has been described in persons of both sexes, and of all ages from 1 year to about 70. It has a world-wide distribution, apparently, but appears to be particularly prevalent in certain countries. Among these may be specially mentioned Sweden, Finland, Russia, and the Baltic provinces generally. Indigenous cases are also reported from Germany, Italy, France,* and the Balkans. The wide distribution of the parasite is shown, however, by recent records of its occurrence in the Ladrone Islands (Prowazek, 1913), the Philippines (Strong, 1904; Walker, 1913*a*; etc.) Java (Brug, 1919*c*), Honduras (Barlow, 1915), Brazil (Axter-Haberfeld, 1915; Pinto, 1919, 1919*a*), and Venezuela (Tagliaferro, 1918; Paez, 1919). No indigenous cases of human infection have yet been reported in Britain, though the parasite is common in British pigs.

Pathology and Morbid Anatomy.—The lesions of balantidiosis, when present, are confined to the large intestine. The parasites cause irritation of the mucous membrane, giving rise to a catarrhal condition, and in more severe cases cause erosion and ulceration. Balantidial ulcers appear, both macroscopically and microscopically, closely similar to those caused by *E. histolytica*. They have now been studied by many workers, and there appears to be no longer any doubt as to the part

* Indigenous French cases have been recently reported by Lanzenberg (1918), Weil and Bergouignan (1919), and Tixier (1919).

played by *B. coli* in their formation. (See especially Walker (1913*a*), who gives an excellent summary of the evidence.)

The earliest change appears to be hyperaemia of the mucosa, often with punctiform haemorrhages. Vascular dilatation, round-celled infiltration, and a local eosinophilia, are also commonly seen. A greater or less degree of erosion and superficial necrosis is usually visible.

Definite ulceration occurs when the parasites penetrate into the tissues. According to Walker (1913*a*) they do this in a purely mechanical manner—displacing the cells of the healthy mucous membrane, and pushing their way into the tissue between the crypts. "The parasites, which are capable of amoeboid* movements, pass between the cells like migrating leucocytes." Later, they multiply in the mucous and submucous tissues, forming nests or colonies. When deep in the wall of the gut, they appear to nourish themselves, like *E. histolytica*, by secreting a ferment which dissolves the cells. Necrosis, with the formation of submucous abscesses and open ulcers, then occurs. In the necrotic areas the balantidia are found peripherally, in contact with the healthy tissues. (Pl. VII, fig. 111.) Sections of balantidial ulcers show, in addition to numerous parasites, coagulation necrosis of the tissues, dilatation of the vessels, round-celled infiltration, and sometimes a localized eosinophilia. Polymorphonuclear leucocytes, when present, probably indicate a secondary bacterial infection. The necrotic tissue closely resembles that seen in amoebic ulcers.

To the naked eye the ulcers appear rounded or irregular, often with undermined edges. The mucosa between adjacent ulcers is frequently hyperaemic. Old ulcers are filled with blackish necrotic tissue, resembling that in the corresponding amoebic lesions.† The presence of the characteristic parasite supplies, in fact, the only means of distinguishing balantidial from amoebic ulcers with certainty.

Balantidia may be found not only in the tissue of the gut wall but also in the blood and lymph vessels in this situation, and even in the lymphatic glands draining the infected areas of the bowel (Bowman, 1909; Walker, 1913*a*). The parasites do not appear to go further into the body: but according to an old and very questionable observation,

* It is unlikely that the movements are truly amoeboid. The "amoeboid" shapes of the parasites in the tissues are probably caused by pressure of the surrounding structures. No ciliates—so far as I am aware—are capable of forming true pseudopodia like anamoeba. (C. D.)

† Excellent figures will be found in the work of Bowman (1909).

they have been coughed up in the sputum—supposedly from a hepatic abscess which had ruptured into the lung (Stokvis, 1884).*

Maliwa and von Haus (1920) have recently published some remarkable observations made upon a young woman at Innsbruck. The patient is said to have passed *Balantidium coli* in immense numbers in her urine.† She suffered from urethritis, cystitis, ureteritis, pyelonephritis of the left kidney, and anuria. On operation the parasites were not found in the kidney, though they were present in the left ureter and the bladder.‡ No suggestion is offered to account for their presence in this singular situation, and unfortunately no examination of the stools appears to have been made. A fuller account of the parasites found—with figures—is to be desired, since this appears to be a unique case.

Marshall (1911) cultivated a ciliate, which he believed to be a *Balantidium*, from the spleen of a patient who died of kala-azar. It measured 42·5μ by 34μ, and from his description it seems clear that the organism was really a free-living ciliate—not *Balantidium*—with which his culture had become accidentally contaminated. It appears probable, also, that contamination with free-living ciliates is the true explanation of the remarkable findings of Hinkelmann (1919), who claims to have found *Balantidium coli* in the peripheral blood of human beings. The same author claims to have found the parasite in the urine also, and to have cultivated it, from the blood, in a medium of blood and water; but his figures and description of the organisms—and others obtained by similar methods—render it highly probable that he was dealing, in reality, not with *Balantidium* but with free-living ciliates from the distilled water employed in the experiments.

Good accounts of the pathology of balantidiosis have been given by Strong (1904), Bowman (1909, 1911), Walker (1913*a*), Brumpt (1913), and Manlove (1917), to whose works the reader may be referred for further details.

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* This case is of interest from another standpoint. The patient, a member of a good family in Holland, has been degraded by the carelessness of bibliographers into "a native of" or "a soldier from" the Sunda Isles: and this extraordinary error is, apparently, the only foundation for the statement almost invariably made—when the geographical distribution of balantidiosis is under discussion—that *B. coli* occurs in that part of the world! Cf. Brug (1919*c*), who has recently directed attention to this mistake, which appears to have originated with Mitter (1891).

† "Es zeigten sich zwischen den Eiterzellen massenhaft Balantidien, die bei wiederholten Untersuchungen zu finden waren" (spaced in original).

‡ The case is complicated by a number of other infections which were found in the lesions (Streptococci, Staphylococci, *Gonococcus*, and *Bacillus coli*).

Symptomatology, etc.—Carriers of *Balantidium* usually display no symptoms. They are not distinguishable from normal healthy persons save by the parasites which they pass from time to time in their stools.

When symptoms of infection are present, they are those of a colitis, with diarrhoea—most commonly—or, in severe cases, an intractable and chronic dysentery. The stools are usually liquid, often contain much mucus, and sometimes blood and pus. Tenesmus and colic are common symptoms, and the colon is usually painful on pressure. Loss of appetite, nausea, thirst, and general debility, are often seen. From ten to fifteen stools *per diem* (or more) may be passed, with much straining and pain. The diarrhoea or dysentery may be continuous or intermittent, and periods of apparent recovery, followed later by relapses, are to be expected: but very often the dysentery, when once established, becomes chronic. The symptoms are said in some cases to resemble those of cholera or typhoid (cf. Krause, 1916). In long-standing cases there is usually emaciation and a secondary anaemia. Eosinophilia has been described,* but it appears probable that the blood-count is usually normal in uncomplicated cases (cf. Bel and Couret (1910), Payan and Richet (1917), etc.). In typical cases, moreover, there is no pyrexia.

Numerous cases of balantidiosis without symptoms have been observed. Walker (1913*a*), for example, states that only 11 out of 57 cases seen in the Philippines displayed symptoms: but he notes that "every person parasitized with *Balantidium coli* is liable sooner or later to develop balantidial dysentery." Pinto (1919) has recently studied 11 cases in Brazil, all without symptoms. In cases which do develop symptoms, the prognosis is not favourable. The disease is usually intractable, and the mortality probably high—29 per cent. according to Strong's (1904) statistics.

It is to be noted that Manlove (1917) has found that, "as in amoebiasis, extensive intestinal lesions in balantidiasis may be present without giving rise to symptoms."

Balantidiosis of Animals other than Man.—Species of *Balantidium* occur in many animals—particularly in Amphibia. The common English frog, for example, harbours three different species in its gut. *Balantidium coli* appears to be able to live in three different

* *E.g.*, Weil and Bergouignan (1919) observed an eosinophilia of 5 per cent. in their patient.

hosts—man, monkey, and pig; and in this it differs from all the other known species. The identity of the forms in these three different hosts was for long in doubt, but recent experiments appear to have settled the matter definitely.

The *Balantidium* of the pig was discovered by Leuckart (1863) in Germany, and identified by him as *B. coli*—the human species, then recently discovered by Malmsten. The *Balantidium* of monkeys was discovered in orang-utans in the Zoological Park in New York by Brooks (1903).* It was later studied in *Macacus cynomolgus*, of Tonkin, by Noc (1908) and Brumpt (1909). The identification of these forms with one another, and with the species found in man, rests chiefly upon the experimental evidence adduced by Brumpt (1909) and Walker (1913*a*). It is now generally admitted that the *Balantidia* occurring naturally in all these hosts are morphologically indistinguishable.

Brumpt (1919) succeeded in transmitting *Balantidium* from monkey to monkey by rectal injection of the active ciliates from the stools. He also passed the monkey's *Balantidium* into young pigs. Finally, he succeeded in parasitizing a monkey with the *Balantidium* naturally occurring in the pig. Brumpt considers that he was dealing throughout with *Balantidium coli*.

Walker (1913*a*) experimentally infected 12 out of 13 monkeys by feeding them with cysts of the *Balantidium* of the pig. Further, he succeeded in infecting 2 out of 4 monkeys by rectal injections of active ciliates from human stools.

It thus appears probable that the same species of *Balantidium* can live in man, monkey, and pig; and the identity of the *Balantidium* of the monkey with that of the pig appears to be proved. Experimental infection of man with the *Balantidium* of either the pig or the monkey has not yet been achieved, however; and it may be recalled that Grassi and Calandruccio (see Grassi, 1888*a*) failed in their attempts to infect human beings by causing them to swallow *Balantidium* cysts from pigs' faeces. They came to the conclusion that the ciliates in the pig belong to a different species from those in man. Nevertheless, direct infection of man by means of the cysts in pigs' faeces has probably been accomplished unintentionally. The evidence is par-

* Brooks also found "*B. coli*" in some "giant turtles from the Galapagos Islands," and he believed that "it was from these animals that our orangs received their infection." But this inference is doubtless incorrect. It is improbable that the *Balantidium* of these reptiles is *B. coli*. (C. D.)

ticularly striking in the case recorded by Young and Walker (1918)—a gut-stripper in a packing factory, who often got pigs' faeces* into his mouth, and became intensely infected with *Balantidium* apparently as a direct consequence. There is also much indirect evidence pointing to the conclusion that man acquires balantidiosis through association with pigs.

Attempts to infect dogs, cats,† rabbits, and other animals with *B. coli* (from human stools) were made by the earlier workers. The results were practically always negative; but the experiments, it must be noted, were not always carried out in a manner conducive to success, and it is possible that more careful work might lead to different results. Experiments which consist in feeding animals upon active ciliates—not on cysts—are doomed to almost certain failure: and from such experiments no satisfactory conclusions can be drawn.

B. coli is usually stated to cause no ulceration in the pig's gut. In Brumpt's experiments, however, one of his experimentally infected pigs showed lesions "identical in every way with those described in man." The observations of Noc, Brumpt, and Walker appear to prove that the monkey behaves towards *Balantidium* in the same way as man—sometimes showing no lesions, but sometimes acquiring a typical ulceration of the colon, accompanied by diarrhoea or dysentery.

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* "The patient stated that he was accustomed to stand ankle deep in hog dung, and that every day he was sprinkled with it and frequently got the fecal material in his mouth." *Op. cit.*, p. 508.

† Behrenroth (1913) states that he succeeded in obtaining a temporary infection in a cat. It is also stated that Casagrandi and Barbagallo were able, by special methods, to infect this animal (cf. Strong (1904), Walker (1913*a*), and others). We have not been able to consult their work on this subject.

CHAPTER VII.

THE DIAGNOSIS OF INTESTINAL PROTOZOAL INFECTIONS.

In previous chapters we have described the Protozoa which live in the human intestine. In the present chapter we shall try to give, with equal brevity, some account of the best methods used for discovering and identifying such organisms, together with practical suggestions regarding methods of collecting and preserving material for examination. We shall also add, parenthetically, a few cautionary hints for the use of the novice who is unfamiliar with protozoology and protozoological methods.

The Collection of Material.—The protozoa living in the bowel are usually studied, of course, in the stools discharged from the body. Since only the cysts of such organisms are able—as a general rule—to live outside the body for more than a few hours, it is of the utmost importance to pay attention to the following points when collecting material for examination.

(1) Stools should always be obtained *as fresh as possible*, and examined *immediately*. As a general rule, stools which are more than a few hours old are unsuitable for examination—except for cysts, which may be found and identified in faeces kept for at least several days, and sometimes for a week or more.

(2) Stools should always be collected in *clean* and *dry* receptacles. It is most important that neither water nor antiseptics should be left in bed-pans or other utensils into which the stools are passed; and care must be taken to insure that urine is not mixed with the faeces. Antiseptics and urine rapidly kill the entozoic protozoa, and water may contain free-living forms which may lead to mistakes in diagnosis.

It is also necessary to prevent foreign particles, such as sand and dust, from becoming mixed with the faeces. This may be difficult under field conditions; but the use of stool-pans with closely fitting lids—only removed for the act of defaecation, and immediately re-

placed—and precautions to prevent toilet-paper, tow, or other cleansing material from coming into contact with the ground, will help to obviate these difficulties.

(3) A *natural stool*—passed spontaneously—should, whenever possible, be obtained for examination. Stools obtained by the administration of purgatives are less suitable for protozoological examination than those passed naturally. If purgatives must be employed, however, salines (*e.g.*, magnesium or sodium sulphate) are the best. Castor oil, and similar substances, should be avoided, as the presence of oil drops in the faeces makes them troublesome to examine under the microscope. Enemas may be useful, but are not usually to be recommended. Noc (1916) advocates rectal injections of thymol for this purpose, and he states that lumps of mucus from the surface of amoebic ulcers (containing *E. histolytica*) can be obtained by this method. Others (*e.g.*, Lyons, 1920) have recommended scraping material directly from the ulcers with the aid of the sigmoidoscope. But such methods are not adapted to everyday use.

(4) Whenever possible the *whole stool* should be obtained for inspection. It should be sent to the laboratory as soon as possible—the receptacle being clearly labelled with the name of the person who passed it, and the date and hour of defaecation. If the stool is to be examined at once, it may be placed in a hot air cupboard—which will preserve the activity of the protozoa for a short time. If the stool must be kept for some time—an hour or two, or possibly days—it should be put in a cold place. Active protozoa and cysts degenerate and perish much more rapidly when warm than when cold.

(5) When the stool has to be sent to a laboratory at a distance, it is usually sufficient to send a sample in a glass or tin tube, such as is now obtainable for the purpose. Glass specimen tubes, measuring about 3ins. by 1 in., and provided with well-fitting corks—into which a glass or metal spoon or spatula has been thrust—answer admirably.* In sending such specimens, it is important to *select a suitable sample.*

* These tubes are fully described in the Report on "*The Laboratory Diagnosis of Acute Intestinal Infections*," published by the Medical Research Council (Special Report Series, No. 51, 1920). It should be remembered that, if such samples are sent by post, in the United Kingdom, they must be carefully packed in metal or wooden cases (hollow wooden blocks are now obtainable for the purpose), securely sealed, and marked "Fragile, with care. Pathological specimen." They must be sent by *Letter Post*—not Parcel Post. (Post Office Regulations.) Failure to comply with these conditions may lead to the official destruction of the specimen and prosecution of the sender.

(And don't forget to label it—a surprisingly common oversight.) When the stool is formed and solid, any part may be sent—a piece about the size of a hazel-nut or walnut being ample. When the stool is uniformly soft or liquid, any portion will do. When partly soft or liquid and partly formed, select a portion of each part. When blood and mucus are present, mixed with faeces, select specimens of each part—if necessary, inclosing the bloody mucous part and the faecal part in separate receptacles.

It is, of course, most important to make sure that the tube is previously *clean* and *dry;* and, especially in the case of liquid stools, that antiseptic fluids have not been left or allowed to dry in it. (With a little practice one can soon learn to select the most appropriate parts for examination from a whole stool. The chief thing to remember is the obvious thing—any part of a homogeneous stool will do, but samples of all the various parts of a heterogeneous stool should be selected for examination.)

Similar precautions should be taken in selecting samples of liver abscess pus for examination. It is important to remember that *E. histolytica* is usually present in the *wall* of the abscess, and not in the first gush of pus obtained on opening it.

Examination of the Stool.—The stool must be examined, of course, under the microscope; but before doing so, it is advisable to make a careful macroscopic inspection, and to record the results.

(A) *Macroscopic Examination.* The following points should be noted. (1) The consistency of the stool or sample—whether hard or soft, formed or unformed, liquid or semi-solid, etc. (2) The colour of the stool. (3) Whether blood, mucus, or pus can be seen by the naked eye. According to the results of this inspection, the stool may be classified as *normal* (brown and formed), *loose* (brown, semi-solid, etc.), *diarrhoeic* (soft to liquid; brown, yellowish, greenish, etc.), and *dysenteric* (loose or liquid, and containing blood and mucus). The presence of any obviously undigested food, sloughs, etc., should also be noted.

(B) *Microscopic Examination.* To make a proper microscopic examination of a stool for the presence of protozoa, a good microscope and accessories are indispensable. The microscope must be fitted with a *mechanical stage*, a *substage condenser*, with rackwork for raising and lowering and an iris diaphragm, and *good lenses*. Three

objectives are almost indispensable — a low power ($\frac{2}{3}$in.), medium power ($\frac{1}{6}$in.), and high power ($\frac{1}{12}$in. oil immersion), and at least two oculars (*e.g.*, No. 0 or 1, and No. 4, 5, or 6). An *ocular micrometer*, which may be permanently fixed in the high-power ocular, and which must be accurately calibrated for each objective and tube-length employed, is also necessary for anything but the most random work.

A good *source of illumination* is also requisite. Artificial light is preferable to daylight for routine work, since it can be kept constant and uniform, and because daylight is usually inadequate for the high-power work that is often necessary. A good electric lamp provided with a screen of ground glass, or an incandescent gas lamp, or even an oil lamp, will suffice : but a better type of microscope lamp is, of course, to be preferred.

Other apparatus, if obtainable, may be necessary or desirable. When it is remembered that it is often necessary to make out with precision the smallest details in complicated organisms or cysts which are smaller than a human red blood-corpuscle, it will be realized that the apparatus just mentioned is the irreducible minimum ; and that for the best work, the best apparatus obtainable, and all the skill and resources of the best microscopist, are not superfluous. (But more mistakes in diagnosis are made as a result of misuse of a good microscope than from the employment of bad apparatus : and nobody who is not accustomed to use a high-power instrument should attempt to diagnose protozoal infections for any purpose but his own diversion or instruction. Experience has proved conclusively that observations made by the inexperienced are practically worthless. The beginner should bear these points constantly in mind, and, as a general rule, should at the outset seek the help and guidance of an experienced and reliable worker. To those with no previous knowledge of protozoology, the task of self-instruction presents almost insuperable difficulties.)

Preliminary Microscopic Examination. The fresh stool should first be examined in such a way that any protozoa which it contains are kept alive and active. To do this, a small portion of the stool is mounted as a thin film under a coverglass. Clean and thin slides should be used, and No. 1 coverglasses. (Large squares ($\frac{7}{8}$in.) are best. Remember that good oil-immersion lenses will not usually work through a coverglass more than 0·140 mm. in thickness.) If the stool

is liquid, a drop may be placed in the middle of the slide with a platinum loop,* and the coverglass carefully lowered on to it and pressed down. If the stool is solid or thick, it must diluted to a suitable consistency before applying the coverglass. This should be done by placing a drop of sterile physiological saline solution (0·75—0·9 per cent. NaCl in distilled water, or Ringer's fluid) on the slide. A particle of the stool is then taken on the platinum loop and emulsified by stirring in the drop. A thin and uniform mixture should be made before the coverglass is applied. (Be careful to prevent the faeces from drying on the loop before stirring into the saline; and do not put the faeces on the slide first and add the saline afterwards. And don't forget to burn off the loop in the flame after making the preparation!) A good preparation made in this way should appear uniform; and the film itself should be free from air-bubbles and so thin that the smallest print is clearly legible through it. (Practice only will teach you how to make the best kind of film.)

As a rule it is unnecessary to seal the preparation (with paraffin or wax, round the edges of the coverglass) or to use a warm stage: though for special purposes these precautions may be necessary. A dark-ground illuminator is sometimes useful, but its use cannot be recommended to beginners.

The preparation should be examined under the microscope in a systematic manner. (Don't push the slide about at random, but begin at one corner of the coverglass and work steadily through from side to side or up and down). It is best to begin with the $\frac{1}{6}$in. objective and the lowest ocular. All protozoa and their cysts can be seen with this combination, though their finer details cannot be made out. When an organism, or other object, requires more detailed study, the oil immersion lens can be substituted for the $\frac{1}{6}$in. (A revolving nosepiece on the microscope is almost indispensable, and greatly facilitates the changing of objectives. Beginners should not try to work quickly. Rapid *and accurate* work is possible for experts only—after long practice.)

Different parts of the stool—collected as described above—should, of course, be examined successively, and the constituents of each part duly noted.

* Under field conditions it is preferable to use long thin sticks for this purpose. A new one is used for each case, and then burnt or thrown into lysol. Wooden matches will also serve in an emergency.

If living and active protozoa, or cysts, are found, they should be examined with the greatest care. Their chief features should be accurately noticed, and an attempt then made to identify them. For this purpose the figures on Plates I and VIII will be of service, as a preliminary step. Afterwards, any special points should be looked up in the descriptive chapters. The determination should then be verified by making preparations in iodine, and, if necessary, permanent fixed and stained preparations.

Iodine Preparations are made in the same way as those in saline solution, but using a watery solution of iodine (1 per cent. iodine in 2 per cent. potassium iodide) in place of the salt solution. (The iodine solution should be fairly fresh. Old solutions lose their efficacy. Emulsify very thoroughly, as the iodine coagulates faecal matter, and cysts and other small objects are therefore difficult to find in badly-mixed preparations.) Iodine kills all the protozoa and their cysts. It fixes them, stains them more or less yellow, and makes their nuclei more clearly visible. The nuclei in cysts can thus be more readily counted, and their structure approximately determined. Iodine has the additional advantage of staining glycogen a deep brown colour—the presence or absence of this material in cysts being a great aid to diagnosis. The flagella of flagellates can also be more easily seen in this medium than when they are alive and moving, and their number, position, and insertion can thus be more accurately determined.

In studying cysts mounted in iodine solution the beginner will find the figures on Pl. VIII helpful. The appearances should be carefully compared with those of the living cysts on the same Plate, and the descriptive chapters consulted for more detailed information.

All wet preparations, after they have been examined, should be thrown into a pot containing lysol or cresol (5 per cent.) in order to sterilize them. The tubes of faeces, when finished with, should be put into a larger vessel containing the same disinfectant—their corks being first removed or loosened—and left there until they can be cleaned again for future use. (The beginner must always remember that cysts are infective, if swallowed : and that even though protozoa are not found in the specimen, pathogenic bacteria may be present. The usual bacteriological precautions should therefore always be observed in handling stools.)

If a fresh film, or an iodine preparation, is examined under a low power with the condenser in focus and its iris diaphragm fully open, too much light will be concentrated on the object. To reduce the light and increase the visibility, the diaphragm should be partly closed, or the condenser racked down. (The latter method—unjustly condemned by some microscopists—is usually the better, and is equally defensible theoretically. The correct adjustment of the illumination can be learned by practice only.)

The foregoing methods of examining fresh stools are essentially those which we, and most of our fellow-workers, always employ.* They are, we believe, the simplest, most direct, and best. Other methods have been advocated, and some of these may now be mentioned. *Direct observation of the living organisms or cysts, however, should never be omitted*—no matter what other methods may also be employed. (It is worth while to spend a long time in examining fresh material, and becoming thoroughly familiar with the appearance of the *living* organisms at all stages of development. Those who are really expert, through long practice, can usually make an exact and rapid diagnosis by this method alone.)

Some workers (*e.g.*, Stitt (1911), Cutler and Williamson (1917), Boeck (1917*a*),† and others) advocate the use of saline solution containing neutral red (1 part in 10,000). This does not kill the active protozoa, and may stain them, and the various objects among which they move, more or less; while cysts remain white, and appear slightly more conspicuous by contrast. Kuenen (1914), Brug (1918), and some of the other Dutch workers, emulsify the faeces with eosin (2 per cent.) for a similar purpose. This rapidly kills most active forms, however, and is not to be recommended for general use. Donaldson (1917) recommends the use of iodine solution combined with a red stain (rubin S or eosin).‡ Cysts appear bright yellow and brown, on a red background, when examined in this medium. This method, however, is merely a substitute for the ordinary iodine method—described above—and will not

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* They were originally described by Wenyon (1915) and have been copied with various modifications by other workers (*e.g.*, Inman (1917), Matthews (1918), etc.).

† This author actually recommends "N/10,000" neutral red solution, but presumably means the concentration given above.

‡ The formula is: 5 per cent. aqueous solution of potassium iodide, saturated with iodine, and mixed with an equal volume of a saturated aqueous solution of rubin S. eosin, or red ink (Stephens's).

enable one to dispense with the examination of the living organisms or cysts also. Methylene violet and methyl violet (Sangiorgi, 1918) and methylene blue solution also have their advocates. Riegel (1918) extracts the azure from Manson's methylene-blue with chloroform, stains coverglass films of faeces in the azure-chloroform solution so obtained, and then mounts and examines them in liquid paraffin.* Amoebae, cysts, etc., are variously stained by this method—and, of course, killed. This, and other methods of rapid staining and fixation (*e.g.*, Mathis's method (1914*a*)—rapid fixation is osmic vapour, followed by staining in haematoxylin solution) are not, in our opinion, to be recommended. If fixation and staining are required, they should be practised with the best cytological reagents.

It should be remembered that there are no true specific stains which will enable one to discriminate any particular protozoon, or its cysts, with absolute certainty. The claims made for some reagents in this respect are not justified. With some stains also (*e.g.*, neutral red), the reaction of the stool may make a considerable difference to the result. (Methods involving drying at any stage—recommended in some of the older medical works—are absolutely useless for the accurate study of any protozoa, and must always be avoided.)

Methods for concentrating protozoal cysts in stools have been devised and advocated by some workers. Cropper and Row (1917) mix the faeces with ether, after emulsification with saline solution; remove the layer of ether, containing the larger faecal particles; spin the saline residue in the centrifuge; and then examine the deposit at the bottom of the tube—in which most of the cysts are collected. This method may be useful for detecting cysts when present in very scanty numbers (cf. also Boeck, 1917*a*, and Carter and Matthews, 1917). In our experience, however, it does not enable one to detect small infections with greater certainty or speed than the direct method here advocated. It has the additional disadvantage of requiring more time, apparatus, and reagents for the various manipulations; and of distorting, killing, or injuring free protozoa and cysts, and so making their identification more difficult.

Carles and Barthélemy (1917) have elaborated a method of concentrating cysts by emulsification, sieving, flotation, and centrifuging—

* For details—discussed with great prolixity—the reader should consult the original.

a method too complicated to be described in detail here. Barthélemy (1917) speaks highly of the results obtained by this method, but we have not tried it.

Methods of counting the cysts present in faeces have been devised by Cropper (1918, 1919) and Porter (1916). Those interested in such methods should consult the original papers—especially that of Cropper (1918), in which his apparatus is fully described.

Permanent Preparations of protozoa in stools can be made in many different ways. We shall describe only the simplest and generally most useful methods. It requires much practice to make really good preparations, and success depends here—as in all other fields of cytology—upon obtaining *perfectly fresh material*, containing *healthy* organisms, upon *proper fixation*, and upon *suitable and accurate staining*. (One of the most important things for the beginner to remember is that the preparations must *never be allowed to dry* at any stage in the proceedings.)

A moist film preparation is made by smearing a little of the material to be mounted—suitably diluted, if necessary, with normal saline solution—upon a clean coverglass ; and then, without allowing it to dry, dropping the coverglass film-side downwards upon the surface of the fixing fluid, contained in a small Petri dish, hollow-ground glass block, or watch-glass. (Hold the coverglass by its edges between the thumb and forefinger of the left hand, and make the smear by carefully spreading the material—as uniformly as possible—with the platinum loop. Thin films are best, but are more apt to dry before they fall on the fixative. If the loop touches the finger or thumb accidentally, wash in lysol immediately. Be careful to avoid the formation of air bubbles between the film and the surface of the fixative. After the film has floated on the surface for a few moments, pick it off with forceps and immerse it completely, face upwards, in the fixative—allowing it to lie thus on the bottom of the vessel until completely fixed. Use plenty of fixative, and throw it away after use. Do not dilute the material too much with saline solution—else the film will not adhere to the coverglass, but will float off and be lost. Films can, of course, be made on slides instead of coverglasses, but this method is less convenient.)

For routine purposes—for fixing both active protozoa and cysts—

the following fixative is the most serviceable (so-called "Schaudinn's solution," with acetic acid):

Saturated solution of corrosive sublimate ($HgCl_2$) in distilled water ... ... 2 parts.
Absolute (or 96 per cent.) alcohol ... ... 1 part.
To every 100 c.c. of the mixture add 5 c.c. of glacial acetic acid.

(This fluid keeps indefinitely—notwithstanding statements to the contrary.)

The film should be left in this fluid for 10 to 20 minutes—the longer time being best for cysts. It is then transferred to 50 per cent. alcohol, in another vessel; rinsed rapidly in this, to remove most of the fixative; and then placed for at least 10 minutes—preferably longer—in 70 per cent. alcohol to which a few drops of iodine solution have been added. This is to remove the rest of the sublimate from the film, before staining and mounting. (Don't forget that the film, after fixation, is soft and delicate, and must never be touched or scratched. Films are most easily handled with fine forceps, with curved ends. Never omit the iodine bath and never use a watery iodine solution. A few drops of the iodine solution used in making temporary preparations—described above—added to about 5 c.c. of 70 per cent. alcohol, answers admirably. Although no sublimate crystals can be seen in the freshly prepared film, they will make their appearance later unless the sublimate is removed in this manner, and will ultimately ruin the preparation.)

It is advisable—if time is no object, and the best preparations are required—to transfer the film from the iodine solution to strong alcohol (70—90 per cent.), and to leave it to harden in this for a day or two. (This prevents maceration or shrinkage during subsequent manipulations.)

Many other good cytological fixatives can, of course, be used. Bouin's fluid, Flemming's fluid, Zenker's fluid, and many others, give excellent results with free organisms; but they often fail to penetrate cysts properly, and cannot be recommended for routine purposes.

Staining may be accomplished successfully in a variety of ways. We recommend Mayer's "Haemalum" for rapid diagnosis of cysts, amoebae, etc., and one of the long iron-haematoxylin methods (such as Heidenhain's) for more accurate work, when speed is not essential, By this method the flagella of flagellates can be stained, and nuclei and other structures can be made to show their finer cytological detail—

neither of these requirements being fulfilled, as a rule, by the haemalum method.

Mayer's "Haemalum" is best compounded as follows :—

Haematoxylin (crystals)	1 gm.
Distilled water	1 litre.

Dissolve and add :—

Potash alum	50 gm.
Sodium iodate ($NaIO_3$)	0·2 ,,

When solution is complete, filter.

(This solution is ready for immediate use. It should be of a rich red colour. It will not keep indefinitely; and when it turns brown and precipitates, it is no longer fit for use. After staining, the solution may be poured back into the bottle, and used again.)

The films to be stained are passed from the strong alcohol, through descending grades of weaker alcohol, into distilled water (*e.g.*, alcohol 70 per cent., 50 per cent., 30 per cent., distilled water. On no account use tap-water.) They are then transferred to the staining fluid, and left there for 5 to 20 minutes. (The longer times are better for cysts, which are less readily permeable than unencysted organisms.) After staining, the films, which now appear pinkish, are placed in running tap-water till blue. (Put them film-side uppermost in a Petri dish containing tap-water, and allow the tap to flow gently into the dish for about 5 minutes or so. If the water is not sufficiently alkaline, it may be necessary to prolong the process, or to add a small amount of sodium bicarbonate or other weak alkali.) They are then ready for mounting.*

The final stages consist simply in dehydrating gradually with alcohol, clearing in xylol, and mounting in Canada balsam. (Pass the coverglasses through ascending grades of alcohol—*e.g.*, 30 per cent., 50 per cent., 70 per cent., 90 per cent.—into absolute alcohol. Leave in this for at least 5 minutes. Then transfer to a vessel containing absolute alcohol and xylol, in equal parts. Leave 5 minutes. Then transfer to pure xylol, when the preparation will clear almost immediately. This slow and gradual method prevents shrinkage and collapse of cysts. To mount, place a drop of balsam—dissolved in xylol—in the middle of a clean and dry slide; then gently lower the coverglass—taken from xylol—film-side downwards on to the drop, and press down

* If the films are found, on subsequent examination, to be overstained, they may be differentiated to the required degree by means of a weak solution of acid or alum.

carefully. Do not use so much balsam that some of it runs on to the back of the coverglass. Be careful not to introduce air bubbles under the film, and not to let the film dry—by evaporation of the xylol—before it is pressed home in the balsam. Harden the balsam, finally, by putting the preparation in a warm but not too hot place—*e.g.*, in the incubator or on the imbedding bath—for a few hours.)

Iron-haematoxylin staining is best carried out as follows. Transfer the fixed films (as before described) to distilled water. Then mordant them in a watery solution of iron alum (2·5—4 per cent.) for not less than 6 hours. (Overnight generally answers well.) Then rinse them in distilled water, and place them in a 0·5 per cent. ripened solution of haematoxylin in distilled water. (Make the solution and put it in a flask, plugged with cotton-wool, in a warm place—if possible in sunlight. Shake from time to time. The solution is "ripe"—*i.e.*, the haematoxylin is more or less oxidized to haematein—and ready for use, when it becomes a good brown colour. This may require several weeks.) The films should be left in this solution for 6 hours or more (up to 24). They will then be overstained and black, and must now be rinsed in distilled water and suitably differentiated by extracting the stain with the iron alum solution (diluted to about 1 per cent.). This is the difficult part of the process, and can only be learned by practice. During the extraction of the stain, the film is removed from the alum solution, rinsed in distilled water, and examined under the microscope. If still overstained, it is put back in the alum, and the process repeated. When the staining is satisfactory, the film is washed first in distilled water, then in running tap-water for at least half an hour. It is then dehydrated and mounted in the manner already described.

Staining can be accelerated by using alcoholic solutions (cf. Dobell, 1914*a*), and by warming. (The beginner should master the other method first.) It is to be remembered, however, that cyst-walls are usually more permeable to watery than to alcoholic solutions; and for this reason various other methods of staining (*e.g.*, with Weigert's iron-haematoxylin,* paracarmine), though often useful, are not so suitable for general use as those just described.

The appearance of the active protozoa or cysts when successfully

* This is a specially useful rapid stain for mucus containing numerous cells or amoebae.

stained by the foregoing method may be gathered from the Plates illustrating this volume. The appearance of stained cysts will be readily understood if the reader will carefully compare the three panels of Plate VIII. The *same cyst*, lying in exactly the *same position*, is here shown* as it would appear (1) when alive, (2) when mounted in iodine solution, and (3) after fixation and staining. Comparison of the figures, with reference to the text, will, it is hoped, obviate the necessity of entering into further detailed description and comparison of the various cysts commonly seen in human faeces. The cysts of each organism have already been described, and their comparison with one another will be facilitated by the figures—which convey more information, if carefully studied, than many pages of printed matter. Nevertheless, the actual appearances of these cysts, and their correct determination, can be learnt only by practical experience in the laboratory; and the few figures which we are here able to give cannot claim to be more than a small and imperfect sample of the almost infinite variety of cysts and other objects which may be encountered in human faeces.

It is frequently desirable—or even necessary—to *counterstain* films, after staining them with haematoxylin by one of the foregoing methods. This is best done with eosin, though any other plasma stain can, of course, be used. (Stain the films to the required degree with a 1 per cent. watery solution of eosin. If overstained, the excess of eosin can be removed by prolonged washing in tap-water, or by dipping in 70 per cent. alcohol containing a very small amount of orange G.)

Very pretty preparations of amoebae and cysts can be obtained by Mann's staining method, as modified by one of us (C. D.). Films are transferred—after fixation, etc.—to distilled water, and then placed for some time (determined by trial—usually 4 to 12 hours) in Mann's stain, prepared as follows:

Aqueous solution of methyl blue,† 1 per cent. ...	35 c.c.
Aqueous solution of eosin, 1 per cent.	45 c.c.
Distilled water	100 c.c.

They are then washed in distilled water, and differentiated in 70 per cent. alcohol containing a little orange G. (A few drops of a saturated solution added to 100 c.c. of 70 per cent. alcohol.) When differentiated

* See the remarks on this Plate in the Preface, p. vii.

† N.B., not *methylene* blue. The methylblue-eosin mixture keeps indefinitely, and may be used over and over again.

to the correct degree (control under microscope), they are dehydrated and mounted in the usual way.

Innumerable other staining methods may, of course, be employed—such as the various carmine and haematoxylin stains, etc.; but the iron-haematoxylin method should be mastered, as it is the only one whereby satisfactory preparations showing the structure of flagellates can be obtained. Borax carmine (especially if warmed, and acidulated with hydrochloric or acetic acid) will sometimes stain cysts when all other stains fail to penetrate their walls.

Glycogen can be preserved and stained in cysts—if permanent preparations are required—by using Best's specific carmine stain for this substance. (See Best, 1906.) Films should be fixed in Carnoy's fluid,* in preference to sublimate-alcohol, though the latter can also be used. They can be stained with Weigert's iron-haematoxylin or any other alcoholic stain before the carmine process, if it is desired to show the nuclei as well as the glycogen.

The technique of preparing sections of tissues infected with intestinal protozoa hardly comes within the scope of the present work. For detailed information the reader must consult the histological treatises devoted to such subjects. We would only remark that for general purposes excellent fixation of protozoa and tissues may be obtained with Bouin's† or Zenker's‡ fluids, while any of the good cytological stains may be employed. The technique of inoculating kittens or other experimental animals with *E. histolytica*, or other protozoa, must also be passed over here. Information on this subject will be found in the work of Dale and Dobell (1917).

It may be added that the cultivation of the intestinal protozoa of man is still too uncertain an achievement for the process to have any value, at present, for diagnostic purposes. References to the successes in this direction claimed by some workers, have already been made in the descriptive chapters.

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* *Carnoy's Fluid:* Absolute alcohol, 6 parts; chloroform, 3 parts; glacial acetic acid, 1 part.

† *Bouin's Fluid:* Formol (40 per cent. formaldehyde), 25 parts; picric acid (saturated watery solution), 75 parts; glacial acetic acid, 5 parts.

‡ *Zenker's Fluid:* Potassium bichromate, 2·5 gm.; sodium sulphate, 1 gm.; mercuric chloride, 5 gm.; glacial acetic acid, 5 c.c.; distilled water, 100 c.c.

Common Sources of Error in Diagnosis.

To deal with this subject adequately would require a whole book. Human faeces may contain innumerable objects, and many of these can be mistaken by the novice for protozoa or their cysts. Since Lambl (1859) first seriously attempted to describe and interpret the microscopic constituents of stools, many works have appeared on this subject. The recent contributions by Cammidge (1914) and Barthélemy (1917) may be mentioned in this connexion. But up to the present there is no work which deals exhaustively with the smaller particles which may puzzle the protozoologist, and we cannot here attempt more than the briefest mention of the most noteworthy of these. A complete descriptive catalogue is out of the question.

It is a wise counsel for the novice that he should never identify any object found in faeces as a protozoon unless he sees it moving. Dead, degenerate, and motionless specimens often cannot be identified with certainty even by the expert. It is wise, moreover, when beginning, never to identify any structure as an amoeba unless it puts out pseudopodia : and never to identify an amoeba, displaying such movements, as *E. histolytica* unless it contains ingested red blood-corpuscles. (If the beginner finds objects which he *thinks* are protozoa or their cysts, but which do not agree exactly with the descriptions or figures, the probability is that he is mistaken.)

When active protozoa are found in a preparation, one should make certain that they have not been introduced in the saline solution with which the faeces have been diluted. To guard against this possibility, the saline solution should be frequently sterilized by boiling, and, if necessary, filtered also. This is especially important in hot countries, and neglect of this precaution is a frequent source of error. It is surprising how many organisms may make their appearance, and even continue to live and multiply, in saline solution or the distilled water used for preparing it. Further, if many active protozoa are found in a stool more than 24 hours old,* it is highly probable that they are coprozoic forms—not intestinal protozoa—which have developed in the stool since it was passed. (See Chapter IX.)

Diarrhoeic or dysenteric stools present the greatest difficulties to the beginner, on account of the numerous cellular elements, derived from

* In hot weather coprozoic flagellates may swarm in stools which are only a few hours old.

the tissues of the intestine, contained in them. The difficulty arises chiefly from the circumstance that these cells, when passed in the stool, are usually in an advanced state of degeneration. Often they in no way resemble the normal tissue-cells with which most medical men are familiar.*

Among the commoner cells which can be mistaken for dead protozoa or cysts we may specially mention the following: detached and degenerating *columnar epithelial cells* and *goblet cells*—often present in mucus from the gut wall, or isolated in the stool; *endothelial cells* from the blood-vessels in inflamed areas—sometimes containing red blood-corpuscles and other inclusions, and thus apt to be mistaken for dead specimens of *E. histolytica*; *squamous cells* from the anal margin; *leucocytes* in pus, or scattered irregularly through the stool. Squamous cells, as seen in stools, often puzzle the beginner, owing to their large size and their resemblance to some pictures of amoebae. Their outlines are often irregular—"amoeboid"—and they possess a clearly visible ring-like nucleus. These cells are usually present on the surface of solid stools, but may be mixed with the faeces in soft or liquid specimens. They can be readily distinguished by their centrally placed nucleus, the small bright granules in their cytoplasm, their lack of motility, and their shape—a flattened scale, not a rounded globule of protoplasm, like a dead amoeba. (The shape can usually be made out by tapping the coverglass, and so causing the cell to turn edgewise.) Polymorphonuclear leucocytes should not give much trouble, as they remain unchanged for a considerable time in stools. Owing to their small size, however, and the apparent presence of several minute annular nuclei in them, they can be mistaken for small cysts of *E. histolytica*—especially when examined in iodine solution.

Worm eggs cannot easily be mistaken for protozoal cysts, owing to their larger size (as a rule), their thick (often coloured and sculptured) shells, and characteristic contents. (The ocular micrometer should be freely used in studying doubtful objects, as their size often gives an important clue to their identity.) Spores of certain *Fungi* may present greater difficulties; and large *yeasts*, fragments of *moulds* (especially in

* It may help the reader to appreciate the difficulties and sources of error if he reads and studies the figures in the papers by Bartlett (1917), and Thomson and Thomson (1916): and then reads the criticism of their findings by Bahr and Willmore (1918). The works by Wenyon and O'Connor (1917), and by Willmore and Shearman (1918), may also be consulted in this connexion.

stale stools), and other colourless living vegetable structures are often mistaken for protozoal cysts by beginners. (The veriest tiro may even mistake oil drops and starch grains—and even air-bubbles—for cysts of protozoa. Rounded homogeneous bodies, derived from the food, and displaying little or no internal structure even when treated with iodine, are common in stools, and should give rise to no confusion. But to determine what some of these structures really are is another matter.) It is impossible to discuss, or even mention, the thousand and one objects—mostly animal and plant remains—of which human faeces are usually composed. They can be learnt by practice only. Their identity can often be guessed by careful inquiry into what the patient has previously eaten, and the guess can then be verified by microscopic examination of the food suspected and by experiment upon oneself. The beginner can learn much by subjecting his own stools to frequent and careful scrutiny—bearing in mind the various foods which he has previously consumed.

The organism which is responsible for the largest proportion of mistakes in diagnosis is probably *Blastocystis hominis*. This is a vegetable organism, probably related to the Ascomycetes (Fungi), and occurs in the intestine of nearly every human being. We give figures of a typical specimen as it appears alive, in iodine solution, and after fixation and staining (Pl. VIII, O^1,O^2,O^3). It consists of a thin layer of protoplasm, containing one or more minute nuclei and a variable number of granules, surrounding a voluminous spherical mass of reserve substance (of unknown chemical composition). Outside the protoplasm there is an extremely thin limiting membrane, and outside this sometimes a gelatinous capsule. Dividing organisms, constricted into an hour-glass figure, are commonly seen. The organism may have any diameter from about $5\,\mu$ to over $30\,\mu$, but such large specimens are very rare. The commonest sizes are from about $8\,\mu$ to $14\,\mu$. The relative proportions of protoplasm and reserve-stuff in different individuals may show considerable variation. Usually the layer of protoplasm is very thin and the reserve mass very large—as in the figures. Sometimes, however, there is a thick protoplasmic layer, and a small mass of reserve material. Other peculiar forms (some of them possibly distinct species) are also encountered.

Blastocystis may be found in the intestine of many animals besides man. It has often been mistaken for the cyst of a protozoon—both in

man and in other animals.* It is a source of trouble to inexperienced workers, and everybody who has to examine human stools should make himself thoroughly familiar with it.

Clinical Interpretation of the Protozoological Findings.

The protozoologist usually examines human faeces with a special object in view. When he seeks for protozoa in a human stool, it is seldom for the mere pleasure of studying these organisms. The stool has usually been passed by a person who is suffering from some intestinal disorder, and the ultimate aim of the examination is to ascertain the "cause" of this disorder. In other words, the protozoological diagnosis is but a contribution to a final medical diagnosis. From the medical standpoint, therefore, the protozoological findings, in a given case, have always to be considered in conjunction with the clinical condition of a patient.

The correct clinical interpretation of the protozoological findings—the correlation of the protozoological and the clinical diagnosis—is obviously a matter of the greatest importance: for upon it the medical diagnosis depends, and upon this will depend, in turn, the treatment and cure of the patient. It is also a matter of immense magnitude. To consider it adequately would lead us to discuss the differential diagnosis of all intestinal diseases. Such a discussion cannot be attempted here; and the few remarks that we can make will therefore be little more than notes.

In order to arrive at the correct interpretation of a given case, the

* Much work has already been done on this curious organism, but its complete life-history has yet to be described. It was first noticed about the middle of last century, and was at one time regarded as the "cause" of cholera. Since then it—or a related species—has been frequently described and almost as frequently misunderstood. It has already been figured as the cyst of an amoeba, of a flagellate, and as a coccidian, in human stools. Prowazek (1904), and most of the German workers, regarded it as the cyst of *Trichomonas*—an organism with which it has no connexion. More recently Chatton (1917) has also supposed that it is a stage in the development of a flagellate—having been deceived, apparently (as Prowazek was before him), by its resemblance to the cysts of *Prowazekella lacertae*. Swellengrebel (1917*a*), on the other hand, regards *Blastocystis* as a degenerative stage of various different intestinal protozoa—in my opinion an equally untenable hypothesis. My own views are given above, and are founded upon a study of *Blastocystis* in many different hosts—a study extending over the last fifteen years, but of which the results are still for the most part unpublished. The generic name *Blastocystis* was introduced by Alexeieff, who has carefully studied the organism (see Alexeieff, 1911, 1911*a*, 1917). With the views expressed in his last publication I am essentially in agreement. I would merely note that he regards the forms occurring in different animals as all belonging to the same species—*B. enterocola*. I consider that there are at least several distinct species, and prefer, provisionally, to distinguish that found in man as *B. hominis*—a name proposed by Brumpt (1912). (C. D.)

following points must always be carefully considered: (1) The *clinical condition* of the patient. (2) The *evidence derived from the macroscopic examination* of the stools. (3) The *protozoological evidence from the microscopic examination*. And in addition to these, we must usually consider (4) other evidence furnished by the *bacteriological examination*, and frequently also (5) the medical *history* of the patient.

We shall, for the sake of brevity, make a drastic simplification of the foregoing programme by eliminating a number of complications which can be entrusted to the common sense of the reader. In what follows we shall assume that the fourth category—concomitant bacteriological evidence—is negative and therefore negligible; and that no other evidence of a like sort (*e.g.*, serological or helminthological) has to be taken into account—an ideal simplification which is rarely or never realized in practice.

We can also simplify the discussion by arbitrary restrictions on the protozoological side. We have already seen, in previous chapters, that there is evidence to show that by no means all the protozoa of the human bowel are pathogenic. All the flagellates, all the amoebae except *E. histolytica*, possibly (or probably) all the coccidia, and all the ciliates except *Balantidium coli*, may be regarded as harmless—at least for present purposes. There is no sound evidence to incriminate them as "causes" of any specific disease. Consequently, their clinical significance will here be regarded as *nil*. If any of these organisms should be discovered—in any stage of development—in the stools of persons with intestinal disorders, their presence can be ignored by the physician; for they probably occur with equal frequency in persons with no such disorders. The clinician may proceed with his diagnosis and treatment as though no such discovery had been made.*

This leaves us with only two protozoa to be considered—*E. histolytica* and *Balantidium*. The former, being much the commoner, is the more important; but what we have to say in the following paragraphs is equally applicable to both, and it must be understood that when we now speak of "parasites," we refer to these two organisms—and these two only. We shall illustrate our remarks by describing, with the utmost

* The propriety of such a procedure has been practically demonstrated over and over again during the recent War. In Britain, at any rate, it was the rule, in the case of military patients, to give a protozoological diagnosis of "negative" to all patients found infected with any protozoon except *E. histolytica* or *Balantidium*.

brevity, three typical cases—from which the interpretation of intermediate or atypical cases can be inferred. The selected types are classified in terms of their clinical condition and the macroscopic appearance of their stools.

Case I. *An apparently healthy person, with formed and normal stools.* In such a person's stools no free (unencysted) parasites will be found. If cysts are present, the person is a *carrier* of the parasite. For closer diagnosis, we must ascertain his history.

If it is found that he has never suffered from dysentery or diarrhoea (amoebic or balantidial*), he is a *contact carrier.* He has not suffered, and probably will not suffer, from the presence of the parasites in his gut. On the other hand, he *may* develop diarrhoea or dysentery—or, in the case of *E. histolytica*, a liver abscess—at any time. The chances are probably remote, but the physician must decide whether specific treatment, to eradicate the infection, is advisable.

If the patient's history reveals the fact that he has previously suffered from dysentery or diarrhoea (amoebic or balantidial), then he is a *convalescent carrier* of the parasite. He has already shown that he is sensitive to the presence of his parasites, and consequently he is liable to a relapse at any time. Specific treatment,† to remove the parasites, is to be recommended—even though the patient is, at the time, apparently in perfect health.

Case II. *A patient who is ill, with diarrhoeic stools.* In the stools of such a patient, we should expect to find free forms of the parasite, probably with a more or less plentiful admixture of cysts. If the patient is suffering from *amoebic diarrhoea*, precystic forms of *E. histolytica* will be present in considerable numbers, while cysts of this amoeba may or may not be present. If he is suffering from *balantidial diarrhoea*, free ciliates will usually be plentiful. Such a patient obviously stands in need of treatment.

Cases of this type often present great difficulty. A carrier can always be made to pass free forms of the parasite which he harbours, and which

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* In practice it will usually be found impossible, when a previous history of dysentery or diarrhoea is elicited, to ascertain the causes of the disease. The physician will usually have to base his decision upon probabilities—taking into consideration the symptoms of the previous attacks, the place where they occurred, the patient's circumstances at the time, the effects of any treatment which he may have received, etc.

† This applies more particularly to *E. histolytica*—the specific treatment of *Balantidium* infections being still problematic (see p. 162).

may be doing him no appreciable harm, by any means (*e.g.*, by a purgative) which will cause him to empty his bowel. Accordingly, a carrier of *E. histolytica*—to take an instance—will probably pass precystic amoebae, of this species, in his stools if he contracts diarrhoea from any cause whatsoever. Consequently, an attack of diarrhoea, with such organisms in the stools, does not necessarily justify a diagnosis of amoebic diarrhoea or dysentery. To justify this diagnosis, other possible causes must be ruled out. The diagnosis is probable if the diarrhoea is *persistent*, and the amoebae are usually *numerous*. It is practically certain if, in addition, the microscope reveals blood or pus in the stools, and active forms of *E. histolytica* containing ingested red blood-corpuscles are occasionally discoverable. The same applies, *mutatis mutandis*, to *Balantidium* infections.

Case III. *A patient who is ill, with dysenteric stools.* In the stools of such a patient, cysts will practically never be found. If active parasites, containing ingested red blood-corpuscles, are present in the stools, a diagnosis of *amoebic* or *balantidial dysentery*—as the case may be—is justified.* Appropriate treatment is therefore necessary.

As a general rule, in a case such as this the parasites will be found in abundance in the freshly passed stools; but they may, occasionally, be difficult to discover. (If the stool is partly faecal, they should not be looked for in this part, but in the flakes or streaks of bloody mucus mixed with it. The faecal part may, however, contain cysts.)

It must be remembered that *negative examinations* may often be made on positive cases: that is to say, an infected person does not always pass his parasites in discoverable numbers in his stools. A negative examination is no proof of non-infection. As a general rule, a negative examination is of less value, as an index of non-infection, in the case of a suspected carrier (Case 1, above) than in the case of a patient displaying acute symptoms (Case 3). When a patient is suffering from amoebic or balantidial dysentery, *E. histolytica* or *Balantidium* can usually be found in his stools without much difficulty. On the other hand, the stools of a healthy person must be carefully examined with

* We have assumed above that other concomitant causes of the condition have been ruled out. It must be remembered, of course, that cases of dysentery due to the presence of two pathogenic organisms simultaneously have been described. Such "double dysenteries" must be extremely rare, however; and even if dysentery bacilli were isolated—for example—from the stools of the hypothetic patient here considered, it would in no way invalidate the diagnosis of "amoebic dysentery" also.

negative results on at least 6 occasions before it can be said with considerable probability that he is uninfected.*

No protozoologist will, of course, ever venture to diagnose a protozoal infection without actually seeing and identifying the particular organism in question. For example, the fact that a patient's condition improved after the administration of emetine would not, in itself, justify the inference that he harboured *E. histolytica*. Indirect methods such as this—which are no more than the making of plausible guesses—can have no place in protozoology. It is true that the stools may have a characteristic look—in typical cases—which may enable one to conjecture the correct diagnosis. The macroscopic appearance of the stools may sometimes enable one to say that the patient is probably suffering from amoebic rather than bacillary dysentery (cf. Grall and Hornus (1914), etc.); but amoebic dysentery cannot be diagnosed with certainty without the aid of the microscope. Further, the microscopic picture of the stools is, on the whole, clearly different in typical cases of amoebic and bacillary dysentery. In the latter the cellular exudate is richer, and contains more numerous leucocytes (cf. Wenyon and O'Connor, 1917; Bahr and Willmore, 1918; Willmore and Shearman, 1918); but to make a diagnosis of "amoebic dysentery" or "*E. histolytica*" from the appearance of the cellular exudate alone—without finding amoebae—would be a highly unscientific procedure.

These and other† indirect methods, which have sometimes been advocated, may have their uses as clinical makeshifts: as protozoological methods they are obviously worthless.

We may be allowed to conclude this chapter with some apt words from an old writer on microscopy—words which are as true to-day as they were when they were written, one hundred and eighty odd years

* The interpretation of negative examinations has been fully discussed elsewhere by one of us (Dobell, 1917). Even more than 6 negative examinations may, of course, be necessary; and this appears to apply especially to *Balantidium* infections—to judge from Walker's (1913*a*) observations on monkeys.

† As a further example, it may be noted that some workers regard the finding of Charcot-Leyden crystals in the stools as evidence of amoebic infection (cf. Acton, 1918). We are not disposed to attach great importance to the presence of these crystals, and will merely note that Barthélemy (1917) considers them to be particularly characteristic of helminthic infections.

ago, and which contain wise counsels that no student of the Protozoa can ever afford to neglect :

"*Cautions in viewing* Objects."

"Beware of determining and declaring your Opinion suddenly on any Object; for Imagination often gets the Start of Judgment, and makes People believe they see Things, which better Observations will convince them could not possibly be seen : therefore assert nothing till after repeated Experiments and Examinations in all Lights and in all Positions.

"When you employ the Microscope, shake off all Prejudice, nor harbour any favourite Opinions; for, if you do, 'tis not unlikely Fancy will betray you into Error, and make you think you see what you would wish to see.

"Remember that Truth alone is the Matter you are in search after; and if you have been mistaken, let not Vanity seduce you to persist in your Mistake.

"Pass no Judgment upon Things over-extended by Force, or contracted by Dryness, or in any Manner out of their natural State, without making suitable Allowances."

—Henry Baker, *The Microscope made Easy*. 1742. Chap. XV, p. 62.

CHAPTER VIII.

THE TREATMENT OF INTESTINAL PROTOZOAL INFECTIONS.

We have now described the chief protozoa which live in the human intestine; and we have also considered very briefly their diagnosis and the pathology and symptoms of the diseases which some of them may play a part in producing. It still remains for us to say something about the treatment of intestinal protozoal infections; that is to say, about the methods of removing these protozoa from the intestine, and so curing a patient of the disease to which their presence may give rise.

In dealing with the subject of treatment, we shall confine our attention almost entirely to one aspect—namely, SPECIFIC treatment. In a work of the present character, dealing primarily with the protozoa themselves, it is impossible to discuss the purely clinical aspects; and for information on this subject we must refer the reader to the ordinary treatises which are devoted to medicine rather than to protozoology. It will be understood, therefore, that when we now speak of treatment, we refer primarily to specific treatment, directed towards the eradication of infection.

We shall discuss, in turn, the treatment of infections with protozoa belonging to each of the four great groups which we have hitherto considered. They will be taken severally and consecutively, because there is no form of specific treatment which is applicable to intestinal protozoa generally. With some of them, such as the amoebae, great advances have recently been made; and as a result we can now eradicate *E. histolytica* from the majority of infected persons with certainty. With most other protozoa, however, the problem is still unsolved. We have no indication, indeed, of the line of attack which we should adopt in attempting to dislodge them from their strongholds in the human body. Consequently, we shall be able to record little but vain attempts at specific treatment in these cases.

The methods of treatment which have been already advocated, and for which success has been claimed, are almost innumerable. Neverthe-

less, but few of these have survived close scientific scrutiny; and in the following pages we propose to consider chiefly those methods which appear to be supported by evidence. Now the evidence necessary to prove that a patient has been "cured" of a protozoal infection is not easily obtained, and a "cure" is by no means so self-evident as one might at first sight suppose. We shall preface the following notes on treatment, therefore, with a few remarks regarding the evidence which is requisite to prove that any particular mode of treatment has been successful.

Let us take an imaginary case. We have a patient suffering from amoebic dysentery—accurately determined by laboratory investigation of his stools, which contain abundant specimens of active *E. histolytica* amoebae, and are bacteriologically and otherwise "negative." We wish to know whether a given drug X is a "cure" for the condition. The patient is put to bed and properly tended, and appropriate doses of the drug are administered. After a few days the patient recovers. His stools gradually become normal, to the naked eye, and his health is restored. In a week or two he is able to resume his ordinary work, and is apparently "cured." Can we, on such evidence, say that the drug X is a "cure" for amoebic dysentery?

The answer to this question, on the evidence so far presented, is not an affirmative—as is sometimes assumed—but in reality an emphatic negative. There is no evidence whatever either (1) that the patient is "cured," in the sense that his infection has been removed, or (2) that the drug X has had anything to do with his clinical recovery. As regards the first point, the disappearance of the infection can be proved only by repeated microscopic examination of the stools, with consistently negative results, for a period of at least several weeks. "Negative" results from naked-eye inspection of the stools, or even a few "negative examinations" made with the microscope, mean nothing; for such negative evidence can often be elicited from untreated cases harbouring many parasites in their intestines. As regards the second point, it must be remembered that amoebic dysentery is often "cured" by rest in bed and nursing. The attack may pass off "spontaneously," without any other treatment. But in all such cases the patient becomes a convalescent carrier of the parasite (see p. 50). He undergoes clinical recovery, but remains infected and liable to relapse at any time.

It is thus clear that we cannot say that the drug X has a specific curative action until, in addition to the clinical recovery of the patient, we have conclusive evidence, from adequate microscopic examination of his stools after its administration, that he is no longer infected with the parasite which caused the disease. (Spontaneous disappearance of the parasites from the intestine has never yet been proved to occur after they have once become established.) It is hardly necessary to add that, for the evidence to carry conviction, there must be no possibility that the original diagnosis was incorrect, and no question of the competence of the protozoologist who made the negative examinations.

Although these requirements may appear self-evident, it is surprising how frequently they have been ignored. Intestinal diseases due to protozoa cannot be diagnosed, nor can their cure be guaranteed, on clinical evidence alone. Consequently, all methods of treatment whose claims to success are unsupported by expert and adequate protozoological evidence, must be regarded with the gravest suspicion.

It is obviously of great importance, in considering the curative efficacy of so-called "specifics" for intestinal infections, to ascertain the proximate value of "negative examinations." An attempt to solve this problem, with sufficient accuracy for practical purposes, has been made by one of us (Dobell, 1917), to whose paper the reader may be referred. The results there reached have received support from subsequent work, and need not be discussed in detail here. It will suffice to notice the chief points of importance.* These are (1) that negative protozoological examinations of the stools made *during* a course of treatment are practically worthless as evidence of non-infection; and (2) that to prove that an infection has been removed by treatment, it is necessary to make at least six negative examinations covering a period of at least three weeks *after* the cessation of treatment. These requirements certainly represent an absolute minimum. Unless they have been fulfilled it is too early to speak of a "cure" having been effected. To arrive at certainty of cure it is necessary to exceed this minimum; and we now consider it desirable to keep all treated cases under protozoological observation for a period of at least a month after treatment.

* The conclusions were based chiefly upon a special case—the treatment of *E. histolytica* infection with emetine. There is good reason to believe, however, that they are equally applicable to the results of treatment of other intestinal infections with protozoa.

If the stools remain consistently negative during this period—the examinations being made every day, or every few days—the probability is that the patient has been freed from his infection.

We shall now consider very briefly the chief results obtained in attempts at specific treatment of the various intestinal protozoal infections of man, and we would ask the reader to bear in mind the points which have just been noted. When we refer to doubtful or inconclusive results, we mean that they appear uncertain because they are not supported by evidence such as we believe to be necessary. If the reader will take the trouble to consult the original works themselves, to which reference is made, he will find that the majority of "cures" rest upon evidence which is far below the standard which we have postulated as a minimum.

The Treatment of Amoebiasis.

In this section we shall deal almost entirely with the specific treatment of primary or intestinal infection with *E. histolytica*. The operative treatment of liver abscess and secondary infections is beyond the scope of the present work; whilst the treatment of most other amoebic infections is still problematic, and also, in practice, unimportant.

There are probably several chemical substances which, when administered to an infected human being, are capable of eradicating an intestinal infection with *E. histolytica*. By far the best known of these, and the most thoroughly studied, is emetine—an alkaloid derived from ipecacuanha. We shall begin with this, therefore, prefacing our remarks with a few notes on ipecacuanha itself.

Ipecacuanha and its Alkaloids.—Ipecacuanha, formerly called "Brazil Root," was introduced into Europe from South America in the XVII century.* It is the root of a Rubiaceous plant, *Cephaëlis* (=*Psychotria* =*Uragoga*) *ipecacuanha*, and was used as a native remedy for "dysentery." It is now known that it has a curative action in amoebic dysentery only, and that this action is due to the indirect specific effect of some of its contained alkaloids upon *E. histolytica*.

* An interesting early account of the use of ipecacuanha for the "Bloody Flux" will be found in the following paper: "*Of the Use of the Root* Ipecacuanha, *for Loosenesses, translated from a* French *Paper: With some Notes on the same, by* Hans Sloane, *M.D.*" (*Phil. Trans. Roy. Soc.*, xx, 69. 1698).

The most important of these alkaloids are Emetine, Cephaëline, Psychotrine, and Methylpsychotrine.*

EMETINE is a powerful gastro-intestinal irritant,† and has a remarkable specific action upon *E. histolytica* in the human body, when administered to the host. Vedder (1912), to whom we owe the revival of its use in recent years, believed, from his experiments with free-living amoebae, that emetine has a specific lethal action upon amoebae generally. There is now good evidence, however, to show that it is not a specially "amoebicidal" substance; and that its action in eradicating *E. histolytica* infections in man is due primarily to its effects upon the host—not upon the parasites directly. (See Dale and Dobell, 1917.) The chief evidence for these conclusions is (1) that its derivatives, and other substances, which are, *in vitro*, far more toxic than emetine to *E. histolytica*, are inefficacious in eradicating human infections; and (2) that emetine will not eradicate an *E. histolytica* infection in the cat.‡ Emetine thus has a specific action not merely upon a particular species of amoeba, but upon that amoeba in a particular species of host.

A derivative of emetine, N-methylemetine, appears to resemble it in its curative action, but is less toxic and less efficacious.§ On the other hand, the stereo-isomeride of emetine called *iso*emetine (Pyman), is comparatively non-toxic to man, but apparently of no value in the treatment of *E. histolytica* infections.‖

CEPHAËLINE is more toxic than emetine, but appears to have similar curative properties.¶ PSYCHOTRINE and METHYLPSYCHOTRINE are comparatively non-toxic, and are therapeutically inactive.**

It appears probable that the toxic action of emetine and cephaëline upon the intestinal mucosa of man is related in some way to their therapeutic efficacy. Non-toxic derivatives, at all events, appear to

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* See especially, on the chemistry of the ipecacuanha alkaloids, Carr and Pyman (1914) and Pyman (1917, 1918).

† On the toxicity and pharmacology of emetine see especially Maurel (1914), Dale (1915), Balfour and Pyman (1916), Johnson and Murphy (1917), Walters, Baker, and Koch (1917), Mayer (1919), Van den Branden (1919), Mattei (1920).

‡ As shown by Dale and Dobell (1917) and more recently by Mayer (1919). Mayer claims some success in treating infected cats with a derivative called "Émetäthylin" (Karrer): but his evidence appears inconclusive.

§ Cf. Low (1915), Stephens and Mackinnon (quoted by Dale and Dobell, 1917, p. 450), Wenyon and O'Connor (1917).

‖ Low (1918), and Low and Dobell (*ined.*).

¶ Cf. Simon (1916), etc.

** On methylpsychotrine see Dale and Dobell (1917) and Jepps and Meakins (1917).

be therapeutically inert. The exact mechanism by which emetine acts upon *E. histolytica* in the body is, however, still a matter for speculation.*

The *mode of administration of emetine* is a point of considerable importance in treatment. The soluble salts of the alkaloid can be administered by the mouth† or subcutaneously.‡ When given orally they generally cause vomiting; but given hypodermically in equivalent therapeutic doses they do not, as a rule, give rise even to nausea. Intravenous injection has been advocated,§ but emetine appears to be more toxic when so given (Dale), and its therapeutic efficacy does not seem to be enhanced. Treatment by intrarectal injection of emetine (or ipecacuanha) has also been employed.‖ Adsorption products of the ipecacuanha alkaloids, or of emetine alone, with fuller's earth ("Alcresta ipecac," etc.) have found some favour,¶ as they cause little or no vomiting. It appears probable, however, that this is due to the contained emetine having been rendered largely insoluble,** and consequently inactive. The results of treatment with such compounds are, at all events, less satisfactory than those which can be obtained by other methods.††

Ipecacuanha itself is still regarded by some workers as superior to emetine. Nevertheless, it appears to contain many unnecessary and inactive ingredients, and its employment seems therefore less scientific than that of its active alkaloids. In our opinion it should be regarded as a substitute for emetine—when this is unobtainable—rather than as a superior compound. It is more emetic, also, and less easily administered.

The experience of the last few years has shown conclusively that the success of emetine treatment depends upon giving *adequate doses* in a *suitable manner*, and on prolonging the treatment for a *sufficient time*. Small doses, given over short periods and intermittently, may

* The fate of emetine, when it enters the human body, is still not known with certainty. It undoubtedly enters the blood-stream, and according to Mattei and Ribon (1917) and Mattei (1920) the greater part of it appears to be eliminated in the urine.

† Cf. Low (1913), Wenyon and O'Connor (1917), etc.

‡ This method was introduced by Rogers (1912).

§ Cf. Van den Branden and Dubois (1915), Van den Branden (1919), etc.

‖ See Lawson (1918), Mayer (1919). The results appear unsatisfactory.

¶ Cf. Allan (1916), etc.

** Cf. Sollmann (1919).

†† See Stephens and Mackinnon (1917) and Donaldson and McLean (1918).

appear to give successful results clinically. They practically never suffice, however, to rid a patient of his infection. Even with larger and continuous dosage, moreover, the method of administration is important. It is now clear, for example, that hypodermic treatment with emetine hydrochloride, in doses of 1 grain daily for 10 to 12 consecutive days, will not radically cure more than about a third of the patients so treated.*

Two methods of administration have hitherto given the best results. These are (1) oral administration of emetine in the form of its double iodide with bismuth, and (2) administration of the hydrochloride orally and hypodermically at the same time. We shall say a few words about each of these methods.

(1) *Emetine bismuthous iodide.* Du Mez (1915) was the first to suggest the employment of the double iodide of emetine and bismuth for the treatment of amoebic dysentery.† He did not, however, make trial of it himself. It was first tried, at the suggestion of Dale (1916), by Maxwell and Paget‡ and Low and Dobell (1916). The results obtained with this drug, *when properly administered*, have been eminently satisfactory.§ It has been subjected to more rigid tests

* See Dobell (1917), Wenyon and O'Connor (1917), etc. The more successful results obtained by this method have invariably been supported by deficient protozoological control of the cases. A large body of evidence in support of the statement made above has been obtained during the War.

† The double iodide of emetine and bismuth is formed by precipitation of soluble emetine salts with Dragendorff's reagent. Precipitation with Mayer's reagent gives an analogous compound, emetine mercuric iodide. Du Mez (1915) prepared both these substances and suggested that they were worthy of trial in amoebic dysentery. He appears to have been unaware, however, that the mercuric iodide had not only been prepared and advocated for a similar purpose by Warden (1891), but that it had even been tested clinically, with favourable results, by Tull Walsh (1891). Warden's reasons for suggesting the use of this compound were, moreover, precisely the same as those of Du Mez. Warden had satisfied himself that "the active remedial agent [in ipecacuanha] is the emetine." And he says: "The most distressing feature attending the treatment of dysentery with ipecacuanha is the deadly feeling of nausea which usually supervenes after the administration of the drug. It seemed possible that if the emetine could be prevented from being absorbed in the stomach, that nausea might be allayed, or, perhaps, wholly prevented." "As is well known, Mayer's reagent is employed as a precipitant for alkaloids from solutions acidulated with sulphuric acid; while the compound of the alkaloid with mercuric iodide is decomposed by alkalies. Theoretically it is possible that an alkaloid in this form of combination would escape decomposition, and hence absorption in the stomach, but be resolved into the free alkaloid and mercuric iodide on coming into contact with the alkaline pancreatic juice." It should be added that Warden's double iodide contained other alkaloids besides emetine, and that it is uncertain how many of the patients successfully treated by Tull Walsh were suffering from *amoebic* dysentery. The mercuric iodide has not been tried in recent years, as it is more toxic than the bismuth compound.

‡ See Dale (1916) and Dobell (1917).

§ See Low and Dobell (1916), Dobell (1917), Jepps and Meakins (1917), Imrie and Roche (1917), Lebœuf (1917), Low (1917), Dobell, Gettings, Jepps, and Stephens (1918), Broc and Chatton (1918), etc., etc.

than any other emetine compound, and has nevertheless given more satisfactory results. We now possess conclusive proof of its curative powers in many cases—some of the patients having been kept under observation (clinical and protozoological) for several years. The failures which have been reported have probably been due, in most cases, to mistakes in the method of administration.*

Since the method of administration is all-important, we may briefly describe the correct method here. The details have been carefully worked out, and various other methods tested, by Dale, Low, Dobell, and other workers in England.

Emetine bismuthous iodide is an almost insoluble brick-red powder, from which emetine is gradually liberated in the alkaline juices of the intestine.† It is therefore important that the liberation of its emetine should not be prevented by combining the drug with insoluble excipients or coating it with substances which do not readily dissolve in the bowel.‡ It has been shown also that compression of the drug into a hard pill or tablet interferes with its action. The drug is best administered pure, as a loose powder enclosed in a hard gelatine capsule or paper cachet. It should be obtained from a trustworthy firm, and be guaranteed to contain not less than 26 per cent. of emetine (alkaloid). The dose should be 3 *grains daily*, by the mouth, *for* 12 *consecutive days*. Shortening or intermission of the course of treatment should not be permitted. Administration of the double iodide in this way usually causes some nausea, but this can generally be mitigated by giving a small dose of opium previously (Tinct. Opii, 10-15 minims), and by giving the drug after the patient has been put to bed—preferably at night, and not on an empty stomach. It is best to give the double iodide in a single dose of 3 grains, and not in separate doses of 1 grain thrice daily.

By the foregoing method, the patient receives, in all, 36 grains of the

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* One of us (C. D.) has had numerous opportunities of verifying this statement. The mistakes are too numerous to mention here, but have generally been due to insufficient dosage, discontinuous treatment, and admixture of the drug with substances which make it insoluble. A large number of published "failures" have been obtained by methods which had already been shown, in the earliest papers published in this country, to be inefficacious. It is remarkable, for example, how many failures have been recorded as a result of a dosage which was shown, in the very earliest trials, to be inadequate.

† See Du Mez (1915), Dale (1916), Sollmann (1919), etc.

‡ For example, excipients such as vaseline, stearin, soap, and resin ointment should not be used; and keratin, salol, stearin, formolized gelatine, shellac, and other more or less insoluble coatings, have all given unsatisfactory results in practice.

double iodide in 12 days. This is usually sufficient to remove an infection with *E. histolytica* permanently, and does not as a rule give rise to symptoms of emetine poisoning. Careful clinical control of all patients during treatment is, of course, necessary. If, after treatment, the protozoological examinations show that the patient is still infected, a further course of treatment should be tried. It is necessary, in this case, to prolong the treatment for a longer period or to administer a larger quantity of emetine each day for a similar period. The patient should therefore be given a double course of the double iodide (3 grains daily for 24 consecutive days), or an ordinary 12-day course together with emetine hydrochloride ($\frac{1}{2}$ to 1 grain daily) hypodermically at the same time.

(2) *Combined oral and hypodermic administration of emetine hydrochloride*, as advocated by Wenyon and O'Connor (1917), has also given very satisfactory results in the treatment of *E. histolytica* infection. By this method a larger quantity of emetine is given than by the double iodide treatment. The dose should be 1 grain emetine hydrochloride hypodermically combined with $\frac{1}{2}$ grain by the mouth—in a keratin-coated tablet—every day for 12 consecutive days. The injection is best given in the morning, and the oral dose after the patient has gone to bed for the night.

Many modifications of the foregoing methods have been adopted—occasionally with apparent success. Some workers still advocate emetine hypodermically combined with ipecacuanha by the mouth—simultaneously or after the hypodermic treatment. It is impossible to discuss these various treatments here; and we must refer the reader to the original works themselves. Among these may be specially mentioned*—in addition to those already cited—the papers by Vedder (1914), Willets (1914), Jones (1915), Lyons (1915), Barlow (1915*a*), Waddell, Banks, Watson, and King (1917), Savage and Young (1917), Noc (1917), Lillie and Shepheard (1917), Watson-Wemyss and Bentham (1918), MacAdam (1919), and Gunn and Savage (1919). It may be added that emetine appears to be well borne by children.†

There is no evidence whatever to prove that there are emetine-resistant strains of amoebae—as is often assumed.‡ There is, how-

* We by no means agree, of course, with all the views expressed by these writers.

† Cf. Archibald (1914), De Buys (1914), Barlow (1914), etc.

‡ *E.g.*, Ravaut (1917), Ravaut and Krolunitski (1917), Mayer (1919).

ever, considerable evidence to show that different human beings may behave differently towards the drug; and that when patients appear to be incurable with emetine, this is because of their own constitution—not that of their amoebae.* There is also some evidence to show that acute cases are more difficult to cure of their infections than carriers.†

Although the majority of persons infected with *E. histolytica* can, apparently, be radically cured of their infections by means of appropriate treatment with emetine, some few patients appear to be quite unaffected by treatment with this alkaloid in any form. Such patients, who are usually sufferers from subacute dysentery, seem to be constituted like the experimentally infected cat—which appears to be incurable with emetine (Dale and Dobell, 1917; Mayer, 1919). For such patients some other treatment is necessary: and although no other drug has yet been proved to be efficacious, successes have been claimed for a number of substances. The most noteworthy of these we shall now briefly mention, since some of them at least merit more extensive trial.

"Chaparro amargosa." This is the Mexican name for a plant called *Castela Nicholsoni*, belonging to the Simarubaceae. It has been used with apparent success by Nixon (1914, 1915, 1916), Shepheard and Lillie (1918), Sellards and McIver (1918), and others, and appears to be worthy of further trial. Another plant in the same family, Simaruba itself, has also some apparent successes to its credit, and has long been known as a "dysentery cure." It has recently been favourably reported on by Yersin, Bréaudat, and Lalung-Bonnaire (1914), Shepheard and Lillie (1918), and Mayer (1919). "Kho-Sam," which is said to be prepared from the seeds of yet another Simarubaceous plant (*Brucea sumatrana*) has also been recommended as a cure for amoebic dysentery (Menetrier and Brodin, 1912; Galliard and Brumpt, 1912; and others). It seems possible that all these plants may contain a common principle of therapeutic value, but at present no alkaloid or other very definite substance appears to have been isolated from them. A crystalline bitter principle has been obtained by Ewins from Chaparro, and a similar (or identical) one

* Cf. Dale and Dobell (1917), etc.

† Cf. Wenyon and O'Connor (1917), Savage and Young (1917), and others.

by Barger from Simaruba*—both of doubtful therapeutic action (cf. Lillie and Shepheard, 1918).

SALVARSAN in various forms has had many advocates, as a "specific" for amoebic dysentery (see especially Ravaut and Krolunitski, 1915, 1916, 1917, etc.). At present we think there is little evidence to show that this substance has a specific action upon *E. histolytica* infections : and the fact that it usually appears necessary to administer it together with emetine, in order to obtain successful results, prevents one from drawing definite conclusions regarding its efficacy.† At present we know of no single case in which it has been proved, by adequately prolonged protozoological control, that an *E. histolytica* infection has been eradicated by salvarsan treatment alone. GALYL has been advocated by Fontoynont (1917), and ATOXYL (*per os*, combined with emetine hypodermically) by Aimé (1917) : but the same reservation must be made in regard to the results which they have published.

BISMUTH salts have been strongly advocated—especially by the workers in Panama (cf. Deeks (1914), James (1918), and others). Proofs of radical cure, by protozoological examination of the stools for a sufficient period, appear, however, to be still wanting. In our experience, bismuth salts alone have no specific action upon *E. histolytica :* and we note that James (1918) now advises the giving of bismuth not alone but combined with "emetine to the point of physiological reaction."

THORIUM salts have been tried by Frouin (1917), who records apparently successful results in a case of amoebic dysentery which had proved refractory to emetine treatment.‡

Among other substances recently advocated we may mention Oil of CHENOPODIUM (Walker and Emrich, 1917; Barnes and Cort, 1918), ADRENALIN (Bayma, 1915, 1917), TANNIN, given hypodermically (Hammacher, 1915), BENZYL BENZOATE (Haughwout, Lantin, and

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* See *Third Annual Report of the Medical Research Committee* (London, 1917), pp. 14, 15.

† Cf. also Noc (1916*a*), who finds that salvarsan cannot be regarded as "a real specific for chronic intestinal amoebiasis." See also Willets (1914).

‡ This treatment appears to merit further trial. The salt used by Frouin was thorium sulphate, 4 to 6 grammes daily *per os*—in a cachet, with food. This treatment was continued for 9 days, and was supplemented during the last 4 days with a daily rectal injection of 200 c.c. of a 2 per cent. solution of the same salt.

Asuzano, 1919), and "Uzara." The last is a proprietory German drug, said to be made from an East African plant (Asclepiadaceae).*

Although emetine—and possibly some other drugs—has so striking an effect upon *E. histolytica* infections, it is somewhat remarkable that it appears to have no action whatever upon infections with most of the other intestinal amoebae. No matter how it be administered, emetine will never eradicate an infection with *E. coli* or *Endolimax nana*;† and no other drug which has hitherto been tried appears to be capable of dislodging these organisms from the human body. There is now evidence to show, however, that emetine, whether given hypodermically or orally, will remove infections with *Iodamoeba bütschlii*.‡ This is the only amoeba, other than *E. histolytica*, upon which emetine has been shown to have any action. How emetine acts in this case is still a mystery, since in its habits and habitat this organism resembles *E. coli* and *E. nana*—which are unaffected by emetine—and not *E. histolytica*.

The Treatment of Flagellate Infections.

It appears probable, from the evidence at present available, that no specific treatment for infection with any species of intestinal flagellate has yet been discovered. This does not mean that claims to such discoveries have not been made: but critical examination of these claims shows clearly that—at least in most cases—they rest upon standards of "cure" which are wholly inadequate.

The appearance of flagellates or their cysts in the stools of infected persons is frequently very irregular. We have numerous records showing that the stools of infected persons may be "negative," on microscopic examination, for considerable periods of time: and this is true of persons undergoing no treatment whatsoever. When "negative" examinations are made during or after a course of "specific" treatment, therefore, they cannot be regarded as evidence of "cure" unless they extend over a period much longer than any "negative period" which may be observed in untreated cases. The figures pub-

* See Waldow and Gühne (1912), Seyffert (1914), Wick (1914), and other German workers, for further details.

† See Dobell (1916, 1917, 1919*a*, etc.), Wenyon and O'Connor (1917), and many others.

‡ See Wenyon and O'Connor (1917), Dobell, Gettings, Jepps, and Stephens (1918), Dobell (1919*a*).

lished by one of us* for *Giardia* may be referred to in this connexion.

Among the "specifics" for flagellate infection hitherto advocated we may mention the following. Mayer (1914) and others have claimed success in the treatment of *Giardia* infection with emetine. We have, however, examined hundreds of patients infected with this flagellate, and others, after treatment with emetine in many different ways; and we can say with confidence that emetine has no effect whatsoever upon infections with any of the common intestinal flagellates.† Another drug which has been advocated strongly by some workers is methylene blue (Castellani (1915), Barlow (1916), etc.); but in our experience it is also without action upon any of the intestinal flagellates. Bismuth salts—*e.g.*, the salicylate—and various "intestinal antiseptics" such as salol, cyllin, β-naphthol, kerol, guaiacol, etc., have also all been equally useless.

Escomel (1913, 1914, 1919), in Peru, claims complete success in the treatment of *Trichomonas* infections with turpentine. He also advocates iodine *per rectum*. Turpentine with us has been worthless (for *Giardia* and other flagellate infections), and this is also the experience of Douglas‡ with *Trichomonas*. Escomel (1917), it may be added, now believes that there are "turpentine-resistant" strains of this flagellate and finds that the same drug will cure bacillary dysentery in the same region (Escomel, 1919*a*).

Recently several workers have recommended salvarsan§ as a specific for *Giardia* infection. Ravaut and Krolunitski (1916) "cured" cases by oral treatment with this drug, but Cade and Hollande (1918) were unsuccessful with this method, and apparently—from the results recorded—equally unsuccessful when they gave the drug intravenously. They remark that their results, as a whole, were "incomplete and variable." More recently, Carr and Chandler (1920) have claimed success, but their results are not supported by sufficient evidence. A number of workers believe that they have been able to cure *Giardia* infections in laboratory animals by means of salvarsan. Thus, Yakimoff, Wassilevski, and

* See Dobell and Low (1916) and Dobell (1917).

† Cf. Dobell (1916, 1917), Wenyon and O'Connor (1917), Dobell, Gettings, Jepps, and Stephens (1918), etc.

‡ See Douglas, Colebrook, and Parry Morgan (1917), where his results are mentioned, but not described in detail. It may be noted that the doses used by Douglas were very much larger than those advocated by Escomel.

§ It may be added that Barlow (1916) found this drug had no effect upon infections with *Trichomonas*.

Zwietkoff (1917) claim to have cured mice of their infections, and Kofoid, Boeck, Minnich, and Rogers (1919) report successful results in treating rats. The evidence presented so far, however, appears inconclusive. We have not ourselves observed any cases of cure by salvarsan in human beings, and regard the reported "cures" with considerable scepticism: and we may add that we have some evidence that salvarsan does not cure *Giardia* infections in the rabbit. One of us (C.D.), some years ago, examined rabbits which had been used for testing various salvarsan preparations, and found that they were all heavily infected with this flagellate, even after fatal doses of the drug had been administered. These observations appear significant, though they are not sufficiently extensive to be conclusive.

Thymol has been advocated by several workers, and Barlow (1916) found it the "most effective" of the drugs which he tried for *Trichomonas* infection. In our experience it has appeared to be without action upon all intestinal flagellates studied.

Other methods of treatment which have been advocated are too numerous and too dubious to mention here. A perusal of the literature on the subject leaves us with the impression—which our own experience confirms—that nothing of any real value has yet been discovered which can be regarded as a specific for the treatment of flagellate infections of the intestine.*

The Treatment of Coccidiosis.

This subject may be dismissed in very few words. No substance has yet been discovered which appears to have any action upon coccidial infections of the human intestine—or, for that matter, upon coccidial infections of any organ of any host. So far as we are aware, nobody has yet made claim to the discovery of any "cure" for coccidiosis.

We will only add that emetine has already been tried, and shown to have no value, in the treatment of human intestinal infections with *Isospora* (Wenyon and O'Connor, 1917; Dobell, 1919; O'Connor, 1919). It seems probable that it is equally useless for the treatment of *Eimeria* infections (Dobell, 1919; Snijders, 1921). The recent observations of

* The "cures" described in numerous papers are also highly unsatisfactory from a zoological standpoint. Many of them refer to "flagellate dysentery" or "flagellate diarrhoea," without specifying the protozoon concerned; and a considerable number of workers claim to have cured infections with "*Cercomonas*" (*e.g.*, Chace and Tasker, 1917). It is impossible to discuss such works without more precise information.

Noc (1920) appear to indicate, further, that salvarsan and thymol are inefficacious for the treatment of *Isospora* infections, though Noc himself considers that they possess some efficacy ("une certaine efficacité").

The Treatment of Balantidiosis.

Balantidiosis is still, unfortunately, a condition for which no really specific treatment has been discovered. Many different drugs have been tried, and successful results obtained with one or other of them have been reported from time to time. But later workers have rarely been able to confirm them. We shall here mention only a few of the attempts at specific therapy—mostly recent, but for the most part not particularly promising.

The older investigators tried quinine (1 in 1,000, or 1 in 2,000), iodine (1 in 10,000), silver nitrate (1 in 3,000), carbolic and salicylic acids, naphthaline, and acetic acid and tannin—all administered rectally—in their attempts to kill the parasites by direct antiseptic action. Successes were occasionally claimed for one or other of these substances, but more often they were found inefficacious. Quinine and calomel *per os* were also largely tried, but on the whole were failures.*

Behrenroth (1913) believed that he had cured a patient by means of de-emetinized ipecacuanha (30-60 pills, containing 0·06 gm. each, daily for 10 days). Barlow (1915) recommends "alcresta ipecac." Axter-Haberfeld (1915) thinks he effected a cure with emetine hydrochloride (hypodermically—0·03 gm. daily for 8 days). Tixier (1919) also believes that his patient was cured with emetine, though from the description it seems improbable that the parasites were eradicated. Brenner (1919) advocates ipecacuanha *per os* (powdered root—1 gm. doses daily for 10-12 days). On the other hand, Dutcher (1915), Lanzenberg (1918), and most other recent workers, have found that emetine has no effect upon *Balantidium* infections.

Barlow (1915) has recommended methylene blue (2 grains, thrice daily, for at least 4 days). Others have not found it of any use. Dutcher (1915) was apparently successful with salvarsan, but Lanzenberg (1918) was not. Labbé (1917) believes he cured a patient by means of rectal injections of silver nitrate (followed by oxygenated water, combined with calomel *per os*), but Lanzenberg (1918) was unable to cure

* For the older methods of treatment see especially Mitter (1891) and Strong (1904).

his patient by this method. Most other workers have had no success with silver nitrate, and the injections are usually extremely painful. Mason (1919) appears to have had some success with oil of *Chenopodium* (60 minims in half an ounce of olive oil, rectally). Lanzenberg (1918) was unsuccessful with thymol, though it was found by Behrenroth (1913) that dead balantidia were passed in the stools as long as this drug was being administered.

Lanzenberg (1918), acting upon Brumpt's suggestions, has recently "cured" a case with somewhat concentrated solutions of quinine, administered *per rectum* (quinine hydrochloride 0·75 gm. in 300 c.c. water). On the whole, this method seems to offer the best chances of success at present. Rectal injections of quinine often appear to relieve the symptoms of balantidial dysentery, and to reduce the number of parasites, even when they do not effect a radical cure.

Haughwout, Domingo, and de Leon (1920) have recently tried benzyl benzoate, and believe that they eradicated the parasites though their patient died—apparently from other causes. Further trials of this drug seem desirable.

Finally, it must be noted that Walker (1913) has tried the effects of a number of substances on *Balantidium* directly, *in vitro*, in an attempt to obtain indications for a specific method of treatment. He found that ipecacuanha and emetine, arsenic and antimony compounds, and various aniline dyes, appeared to have but little action on the parasite. Mercury and silver salts, on the other hand, appeared to be "balantidicidal"—the organic compounds appearing more potent than the inorganic. It seems doubtful, however, whether experiments of this sort will afford any real indications for treatment.*

* Compare, for example, the observations of Dale and Dobell (1917) on *E. histolytica*. In the case of this parasite, it seems clear that the relative toxicity of various substances *in vitro* is no measure of their therapeutic efficacy *in vivo*. Analogous instances could also be quoted.

CHAPTER IX.

THE COPROZOIC PROTOZOA OF HUMAN FAECES.

It has already been noted in the Introduction (p. 16) that there are certain protozoa which are sometimes found in human faeces, but which are not entozoic. Such organisms are suitably designated Coprozoic Protozoa. This name does not, of course, denote a natural group in the zoological system, but merely refers to a habit of life common to a number of organisms belonging to various groups.

The coprozoic forms which may be met with in human faeces are, for the most part, forms which occur in nature in the faeces of other animals also, and in organic infusions of various sorts. They belong to the Rhizopoda and Mastigophora,* and only a few of them can be said to be common. In this final chapter we shall notice merely the commonest or most interesting organisms.

Coprozoic protozoa may gain access to faeces in two different ways. As they are all free-living organisms, capable of living, as a rule, in water or decomposing organic matter of many kinds, they may contaminate human excrement after it has been discharged from the body : and if the conditions are suitable, and they find this material a favourable culture medium, they may continue to live and multiply in it just as they would in any other decomposing matter. On the other hand, coprozoic protozoa frequently get into human faeces by another route. Their cysts, carried by air, water, or other means, may be swallowed by a human being with his food or drink. They then pass through the alimentary canal with the food, and are finally discharged with the stools. If they have thus survived the passage through the body, they excyst in the deposited faeces and proceed to multiply in this material. Such organisms are usually incapable of living in human faecal matter

* It is doubtful whether any truly coprozoic Ciliata occur in human faeces, though free-living species of this group may sometimes get introduced accidentally. Cf. pp. 113-117 *supra*.

while it is still within the body—probably because the temperature is too high and oxygen is lacking.*

This remarkable ability to pass, in the encysted state, safely through the body of an animal, without undergoing any development, was long an unrecognized property of some of the small free-living amoebae and flagellates; and ignorance of it has led, in the past, to many mistakes. It is now easy to understand how it is possible to find typically free-living species of protozoa swarming in faeces shortly after leaving the body—notwithstanding the fact that the faecal material may have been carefully collected in sterile vessels and guarded from contamination from outside. Although some of the older workers had reached correct conclusions on this subject,† the facts were first put on a scientific footing by the experiments of Walker and Sellards (1913) with free-living amoebae.

Many of the coprozoic amoebae and flagellates found in human faeces have been described as human "parasites," and they have often been named as "new species" owing to their identity with well-known free-living forms having been overlooked. The literature on many of these free-living forms—described as such—is also in a very confused state at present, and bristles with mistakes and uncertainties of all sorts. Consequently, it is by no means easy to deal with this subject accurately and briefly. It is, in fact, quite impossible to attempt an exhaustive survey of these organisms within the limits of one short chapter, and the following few pages make no pretentions to completeness. It is hoped, however, that they will serve as an accurate introduction to the subject, and will enable the reader, desirous of pursuing it further, to find his way to the more important work which has hitherto been done.

(A) Coprozoic Rhizopods.

The commonest coprozoic Rhizopods found in human faeces are the small amoebae commonly, but incorrectly, called "*limax* amoebae," or "amoebae of the *limax* group." Of these there are many species, but their identification is difficult and their classification is at present in a well-nigh hopeless muddle.

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* This is inferred from their behaviour in cultures: *e.g.*, *Bodo caudatus* will not grow at 37° C., or in the absence of free oxygen. (C. D.)

† For example some of the Italian workers—especially Casagrandi and Barbagallo (1897*a*).

The name "*Amoeba limax*" was given by Dujardin (1841) to a fresh-water "species" which is not now identifiable.* The most that can be said about it is that it was certainly not any one of the forms which are now generally called "*Amoeba limax*;" since these are all very small, and Dujardin's "species" is stated to have measured 100 μ by 30 μ. Instead, therefore, of attempting to fix this name upon any of the different species which have since been described, it would be much better to drop it altogether.

Many of the small free-living amoebae are so much alike in their active amoeboid stages, that it is impossible, at present, to distinguish them from one another by these stages alone. It is necessary to know the whole life-history, or at least the chief stages. For purposes of identification a knowledge of the following characters, at least, is indispensable: (1) The structure and size of the free amoeboid form (especially the finer details of nuclear structure); (2) all the chief stages of nuclear division; (3) the size and structure of the cysts (cyst-wall, number of nuclei, inclusions—such as chromatoid bodies, etc.); (4) other stages of development, if they occur—especially the presence or absence of a flagellate form. Endless confusion has already been caused by the renaming of species previously named—either through ignorance of previous work or through failure to recognize a described form owing to malobservation or misdescription; by describing and naming a few stages in the life-history, when other stages had already been described and named; and by introducing new specific names for organisms which can never subsequently be identified—owing to the author's omission to record (and often even to observe) any characters of real systematic value.

A large number of free-living amoebae obtained from human stools have already been observed, and some of them named—for example, by Celli and Fiocca (1894), Musgrave and Clegg (1904), Walker and Sellards (1913), Whitmore (1911*a*). The majority—probably all—of these are not now identifiable with any certainty. The same is true, unfortunately, of the greater number of the small species of free-living

* It is worthy of note—since it is usually overlooked—that Dujardin himself did not profess to be able to distinguish his "species" of *Amoeba* by any good specific characters. His names were nearly all of uncertain application and doubtful validity. He says himself (1841, pp. 231-2) that the characters which he employed for purposes of classification "are by no means true specific characters"; and he especially adds that in his "enumeration" of various forms, under different names, "*il est donc bien essentiel de ne pas voir une distinction d'espèces.*" (C. D.)

amoebae obtained from other sources. We have studied many species during the last 15 years, but have been able to identify and name but few. In the case of the amoebae from human stools we have therefore selected three of the most certainly determinable species which we have studied, and give them here—with sufficient detail for their recognition by others—merely as types or examples of the forms which may occur. The species selected belong to three different genera—*Dimastigamoeba* Blochmann, 1895 (*emend.* Alexeieff, 1912*c*), *Hartmannella* Alexeieff, 1912*a* (= *Hartmannia* Alexeieff, 1912), and *Sappinia* Dangeard, 1896 (*emend.* Alexeieff, 1912*e*). These three genera are now readily identifiable by their nuclear structure, nuclear division, and the characters presented by their cysts. *Dimastigamoeba* is further characterized by possessing a flagellate form.

These organisms will now be briefly described, and our account of coprozoic rhizopods will then finish with a note on the interesting shelled amoeba *Chlamydophrys*. Before giving descriptions of these amoebae, however, a word may be said about their cultivation.

Cultivation.—Most of the small amoebae which live in water, soil, infusions, faeces, etc., can be readily cultivated. Most of them feed upon bacteria, and must therefore be cultivated together with these micro-organisms—to serve as food. Much has been written on this subject; but it will suffice to refer to the well-known works of Musgrave and Clegg (1904), Walker (1911), Walker and Sellards (1913), and Wülker (1911), where fuller references to the literature will be found.*

The small coprozoic amoebae can be cultivated on solid media (agar, etc.) or in liquids (hay infusion, dilute egg-albumin, etc.). One of the most useful media is Walker's modification of Musgrave and Clegg's "amoeba agar," prepared as follows :†

Agar	2·50 gm.
Sodium chloride	0·05 ,,
Liebig's beef extract	0·05 ,,
Normal sodium hydroxide solution	2·00 c.c.
Distilled water	100·00 ,,

(Sterilize in autoclave. After sterilization, reaction approximately neutral.)

* See especially the useful resumé of Wülker (1911).

† See Walker and Sellards (1913), p. 265.

Amoebae grow best on media containing plenty of water, or in a moist atmosphere. For this reason it is a good plan, after the inoculation of an agar plate with faeces or other material containing amoebae, to invert the Petri dish and pour a little water in the lid. In many liquid media—such as 5 per cent. egg-albumin solution—amoebae often thrive wonderfully. It should be remembered that in such cultures the organisms are usually present in the surface film or on the sides and bottom of the vessel. (Amoebae—unless they possess a free-swimming flagellate stage—can only creep on a more or less firm surface. They are unable to swim in a liquid.) In old cultures the amoebae encyst, but the cysts usually hatch readily on transference to new medium. Cultures can thus be kept going indefinitely; or the cysts can be kept for months, or even years, and used to prepare new cultures at any time by merely sowing them in fresh medium.

(1) *DIMASTIGAMOEBA GRUBERI* (Schardinger) Alexeieff, 1912.

Chief synonyms:

Amoeba gruberi Schardinger, 1899.
Amoeba diplomitotica Aragão, 1909.
Amoeba punctata Dangeard, 1910.
Vahlkampfia punctata (Dangeard) Chatton & Lalung-Bonnaire, 1912.
Amoeba tachypodia Gläser, 1912.
Naegleria punctata (Dangeard) Alexeieff, 1912.
Vahlkampfia soli Martin & Lewin, 1914.
Naegleria gruberi (Schardinger) Wilson, 1916.
Wasielewskia gruberi (Schardinger) Zulueta, 1917.

This amoeba is one of the most easily recognized of the species which may occur coprozoically in human faeces, from which it was first obtained by Schardinger (1899) in Vienna. It has often since been studied—usually from soil—and almost as often renamed. The foregoing list of probable synonyms is not complete, but indicates the chief names under which the organism has previously been described. The most detailed account which has yet appeared is that of Wilson (1916), to whose work the reader is referred for a more complete description than is here possible.

The active AMOEBA of this species (Pl. IV, fig. 49) is small, measuring as a rule from about 7μ to 15μ in diameter when rounded. Each animal possesses a single vesicular nucleus, about 3-4μ in diameter, with a large central karyosome and rather sparse granules of "peripheral chromatin" in the clear zone between it and the nuclear membrane; and each amoeba also has a single contractile vacuole, formed by the fusion of several smaller ones. During locomotion the amoeba usually displays several large pseudopodia, composed chiefly of clear ectoplasm, at its anterior end. The endoplasm contains food vacuoles enclosing ingested bacteria.

The NUCLEAR DIVISION of this species shows a number of very characteristic mitotic figures—one of which (equatorial plate stage) is shown in fig. 51* (Pl. IV). The process has been studied in detail by Gläser (1912), Ford (1914), Wilson (1916), and others.

One of the most striking characters of the species is its ability, in certain circumstances, to assume a free-swimming FLAGELLATE STAGE (Pl. IV, fig. 50). The amoeba contracts into an oval shape, and its nucleus takes up a position at the more pointed anterior end. From the anterior pole of the nucleus two long flagella develop, of equal length and both directed forwards. They appear to grow out of basal granules situated on the nuclear membrane. In these flagellate forms the contractile vacuole always occupies a posterior position (fig. 50). The amoebae can usually be made to assume the flagellate condition by simply flooding the culture with an excess of water. After a variable time the flagellate forms lose their flagella and again become amoebae.

A similar flagellating amoeba has been described from human faeces by Whitmore (1911*a*), and named by him *Trimastigamoeba philippinensis*. It appears to differ from *D. gruberi* only in having—according to the description—3 flagella instead of 2 in its flagellate stages.

The CYSTS (Pl. IV, fig. 52) are spherical structures, measuring 8-12μ in diameter—their average being about 10μ. They are uninucleate, and when first formed contain numerous rather large, spherical, deeply staining chromatoid bodies. Their walls are double—the outer layer being the thicker, and presenting a variable number (usually 3-8) of

* Note the "polar caps" of chromatin, and the persistence of the nuclear membrane. Cf. fig. 54, of *Hartmannella*.

pores, each of which is surrounded by a slight thickening of the cyst wall.* The amoeba emerges through one of these pores during excystation, and the pores themselves are most clearly visible in empty cysts. They are characteristic of this species.

D. gruberi appears to be one of the commonest species of the small free-living amoebae. It probably occurs in soil and water almost everywhere, and is easily cultivable in hay and soil infusion, in diluted egg-albumin, or on agar plates (see p. 167).

(2) *HARTMANNELLA HYALINA* (Dangeard) Alexeieff, 1912.

Synonyms :

Amoeba hyalina Dangeard, 1900.

? *Amoeba hyalina* (Dangeard) Brodsky, 1910.

? *Amoeba hyalina* (Dangeard) Hartmann & Chagas, 1910.

The amoeba here described under the above name is not referable to Dangeard's species "*Amoeba*" *hyalina* with absolute certainty. It is also doubtful whether the organisms referred to the same species by Brodsky (1910) and by Hartmann and Chagas (1910*a*) are identical either with Dangeard's species or with ours. It is not improbable, however, that all belong to the same species, since it is one which appears to be common and widely distributed. As regards the generic name there is more certainty, since the amoeba in question appears to be undoubtedly a member of the genus originally named *Hartmannia* by Alexeieff (1912) but subsequently changed by him to *Hartmannella* (1912*a*)—the former name being preoccupied.†

It is probable that the organism obtained in cultures from liver-abscess pus, air, and water by Wells, in India, and described by Liston and Martin (1911) and Martin (1911) as the "large amoeba from liver abscesses," really belongs to this same species. It is also likely that it is identical with one of the species of amoeba cultivated from human faeces by Whitmore (1911*a*), and collectively designated by him "*Amoeba limax* subspecies M. II." The organism has also probably been obtained from similar sources by others.

* Two of these pores are shown (in optical section) in the wall of the cyst depicted in fig. 52.

† Alexeieff (1912*a*) has named *H. hyalina* Dang. as the type, but has not amended the diagnosis by any adequate redescription of the actual organism. And it is still doubtful to what animal the name was originally given—the observations of Dangeard being far from complete. (C. D.)

This organism differs considerably from *D. gruberi*. The amoeboid form is closely similar, but the method of nuclear division is entirely different. Moreover, it possesses no flagellate stage,* and its cyst is larger, and has a thick crinkled wall. (There are many other closely related species with similar characters.)

The AMOEBA (Pl. IV, fig. 53) is usually slightly larger than *D. gruberi*, measuring from about 9 μ to 17 μ in diameter when rounded. It posseses a single contractile vacuole. Its nucleus is closely similar to that of *D. gruberi*—and of most other small amoebae—and consists of a spherical vesicle with a large central karyosome, and somewhat abundant "peripheral chromatin" granules in the clear zone.

MULTIPLICATION occurs in the usual way by fission into two. The stages of nuclear division are highly characteristic. The division is a typical mitosis,† with the formation of a sharply pointed achromatic spindle, and tiny spherical chromosomes (Pl. IV, fig. 54). The nuclear membrane disappears during the process, and there are no "polar caps" of chromatin, and no connecting chromatin strand is present in the telophases—as in *D. gruberi*.

The CYSTS (Pl. IV, fig. 55) are uninucleate, and double-walled. They usually measure from 10 μ to 14 μ in diameter. The inner wall is thin and smooth, the outer—when fully formed—very thick, wrinkled, and brownish in colour, with no pores. Small spherical chromatoid bodies are present in newly-formed cysts, and are sometimes very abundant. As in other species, these bodies disappear in older cysts.

This species is readily cultivable on agar (see p. 167) and in many other media.

It may be added that the process of "endogenous bud-formation" described in this species (?) by Liston and Martin (1911) has never been observed by us: but we have seen the phenomenon so interpreted in other species, and believe that the "internal buds" are merely small amoebae of a different species which have been ingested as food. We believe there is no good evidence of reproduction by internal budding in any of the small amoebae.

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* Numerous attempts to obtain flagellate forms by the methods successful with *D. gruberi* have always been completely negative. (C. D.)

† I have studied all stages, but it is impossible to give a complete series of figures here. (C. D.)

(3) *SAPPINIA DIPLOIDEA* (Hartmann & Nägler) Alexeieff, 1912.

Synonyms :

Amoeba diploidea Hartmann & Nägler, 1908.
Vahlkampfia diploidea (Hartmann & Nägler) Calkins, 1912.

We follow Alexeieff (1912*e*) in referring this very interesting amoeba to Dangeard's genus *Sappinia*. Its generic designation is still, however, open to question (cf. Chatton, 1912). We have seen the organism but rarely as a coprozoic inhabitant of human faeces, and it appears to occur more commonly in the excrement of several other animals (lizard, ox, etc.).

The AMOEBAE (Pl. IV, fig. 56) are of moderate size, measuring some 10-30 μ when rounded and at rest. They usually display but slow movements. Their distinctive characters are the possession of a comparatively thick, though smooth and hyaline, skin or pellicle—sometimes more or less wrinkled, as in "*Amoeba*" *verrucosa* (or "*A.*" *terricola*)—and two nuclei. These nuclei are identical in structure, and usually closely apposed. They are vesicular, and each contains a large central karyosome surrounded by a clear zone containing "achromatic" granules. A single contractile vacuole is present, but it pulsates very slowly.

The life-cycle has been described by Hartmann and Nägler (1908) and Nägler (1909), but certain points in it require further elucidation. These authors obtained their material from the excrement of lizards.

MULTIPLICATION is effected by division into two—the two nuclei undergoing mitosis simultaneously, side by side. The daughter individuals are thus binucleate from the moment of their birth (cf. *Dientamoeba*, p. 37).

The CYSTS of this species are very remarkable structures (Pl. IV, fig. 57). Before encystation, two individuals come together; and after creeping round one another for some time, they form a single cyst in common. Newly formed cysts thus always contain two individuals, in close contact. The cysts themselves are spherical, with fairly thick but uniform walls, and measure from about 12μ to 18μ in diameter. According to Hartmann and Nägler, a remarkable sexual process takes place inside the cyst. The two nuclei first fuse in each individual, so that the cyst comes to contain a pair of uninucleate amoebae. "Reduction"

phenomena are then said to occur, after which the two individuals fuse. Only their cytoplasm fuses completely, however, their nuclei coming in contact, but remaining separate. The cyst thus contains, at this final stage, a single binucleate individual. When the cyst hatches later, this individual emerges and begins life anew as the ordinary binucleate free form. It will be noted that, if this account is correct, the nuclei of the free forms must be regarded as unfused gamete nuclei from a previous incomplete conjugation.

This account still requires confirmation, and we are by no means certain, from our own observations, that the foregoing interpretation is correct. It appears certain, however, that two binucleate individuals enter into the formation of each cyst, and that only a single binucleate form ultimately emerges from it.

Like the other coprozoic amoebae, *S. diploidea* is easily cultivable on agar (p. 167).

(4) *Chlamydophrys stercorea* Cienkowski, 1876.

Synonyms:

Troglodytes zoster Gabriel, 1876.
Platoum stercoreum (Cienkowski) Bütschli, 1880.
? *Leydenia gemmipara* Schaudinn, 1896.

Chlamydophrys is one of the shelled amoebae (Thalamophora or Thecamoebae), and differs considerably from the naked rhizopods previously described. We have never succeeded in finding this organism in human faeces, though we have looked for it innumerable times: but according to Schaudinn (1903) it is very common in this situation. One of us has studied it, however, in the faeces of frogs and toads (Dobell, 1909)—the figure here reproduced having been drawn from a specimen found in the excrement of one of these animals (*Bufo vulgaris* L.).

The organisms which we have studied (Pl. V, fig. 96) possess oval shells, measuring, in well-grown individuals, about 20 μ by 14 μ. The shell itself is thin, white, and smooth, resembling porcelain. It has an opening at its more pointed end, through which the protoplasm and pseudopodia project in the living animal. The pseudopodia are filose and sometimes branched, and serve to capture food. There is a single large vesicular nucleus in the dense protoplasm at the opposite (closed)

end of the shell. It possesses a voluminous and deeply stainable spherical karyosome. The protoplasm is much vacuolated towards the more pointed end, and sometimes contains one or more contractile vesicles.

In younger stages the animal is devoid of a shell, and closely resembles the so-called "*limax*" amoebae. It creeps about in an amoeboid fashion, is able to encyst, and can probably reproduce by fission in this form. The shelled forms multiply by the process of "budding division" characteristic of shelled rhizopods generally. They are also able to encyst—their cysts being uninucleate, and furnished with very thick, irregular, and brownish or yellowish walls.

Schaudinn (1903) made some remarkable statements concerning the life-history of *Chlamydophrys*, and briefly described its division, conjugation, etc. Figures of the various phases originally described were published later in his posthumous works (Schaudinn, 1911), but his descriptions are still unconfirmed and not sufficiently detailed to carry conviction. Some of his statements, indeed, are almost certainly incorrect. He stated, for example, that it is necessary for the cysts to pass through the intestine before they can hatch in human faeces, and that sometimes they even hatch in the intestine, where the organisms are able to live and multiply as naked amoebae. He claimed to have found these amoebae in perfectly fresh human faeces, but nobody has yet confirmed this observation,* and we have never succeeded in finding them. It should be remembered, in this connexion, that Schaudinn was not acquainted with several of the species of amoebae living in the intestine of man, and held incorrect views about the development of the two species which he did know.

But the most remarkable statement made by Schaudinn (1903) is that "*Leydenia gemmipara*" is an abnormal amoeboid form of *Chlamydophrys* which has gone astray in the peritoneal cavity. There is good reason to believe, however, that "*Leydenia*," described by Leyden and Schaudinn (1896), is not an amoeba at all. The "amoebae" were probably cells belonging to the human body (cf. Dobell, 1919*a*). At all events, no confirmation of Schaudinn's extraordinary assertion has hitherto been forthcoming, and it is still unsupported by any evidence.

* The "*Chlamydophrys*" amoebae found by Elmassian (1909) were probably *Endolimax nana*. Cf. Dobell (1919*a*), p. 135.

It should be noted that Schaudinn's posthumous figures of *Chlamydophrys* differ in some respects from those of other workers. The shape of the shell, for example, differs from that shown in our figure (Pl. V, fig. 96)—the shell, in Schaudinn's specimens, being drawn out into a neck at the pointed end, with the margin of the opening everted, so that it is flask-shaped rather than oval. The dimensions are not stated. It thus seems not improbable that Schaudinn's form belongs to a different species from that which we have studied.

(B) Coprozoic Flagellates.

(5) *Bodo caudatus* (Dujardin) Stein, 1878.

Chief synonyms :

Amphimonas caudata Dujardin, 1841.
Bodo urinarius Hassall, 1859.
Diplomastix caudata S. Kent, 1881.
Bodo asiaticus Castellani & Chalmers, 1910.
Prowazekia cruzi Hartmann & Chagas, 1910.
Prowazekia weinbergi Mathis & Leger, 1910.
Prowazekia asiatica (Castellani & Chalmers) Whitmore, 1911.
Prowazekia javanensis Flu, 1912.
Prowazekia urinaria (Hassall) Sinton, 1912.
Prowazekia italica Sangiorgi & Ugdulena, 1916.

This is the commonest of all the coprozoic flagellates found in human faeces. It is also very common in organic infusions of many kinds. The organism named *Bodo urinarius* by Hassall (1859), and found by him in human urine, almost certainly belongs to this species—as is evident from the more recent account of it given by Sinton (1912).* Almost every worker who has studied this organism seems to have given it a new name, so that the above list of probable synonyms is by no means exhaustive. It may be added, however, that many of the published accounts are not sufficiently precise for it to be possible to identify the described organisms with absolute certainty.

The genus *Bodo*, originally proposed by Ehrenberg, has often proved a puzzle to protozoologists; but as a result of the work of Klebs (1892),

* Woodcock (1916) is of the same opinion.

Stiles (1902), Alexeieff (1911*b*, 1911*c*, 1912*d*, etc.), Kühn (1915), and others, it may now be regarded as definitively established. The following are its distinctive characters: The organisms are all small, more or less elongate or oval, and possess two flagella, both arising at the anterior end—one directed forwards, the other trailed behind. There is a vesicular nucleus, with a large central karyosome, near the middle of the body. A small permanent mouth is present at the anterior end, and near it is a minute contractile vacuole. Furthermore, at the anterior end of the body, and closely associated with the roots of the flagella, there is a conspicuous rounded and deeply stainable body. This structure is usually called a "kinetonucleus," and is homologous with the structure to which the same name is applied in the Trypanosomes (so-called "blepharoplast" of German writers—though not a blepharoplast proper). We consider that this structure is not a nucleus, but homologous with those bodies in other flagellates—of doubtful function—to which Janicki (1911) has given the name "parabasals." We shall adopt the name "kinetoplast" proposed for it by Alexeieff (1917*a*). All species of *Bodo* multiply by simple longitudinal fission, and form oval cysts containing a single individual (as a rule).

B. caudatus displays the following features. The active FLAGELLATES (Pl. V, figs. 78-80) are polymorphic, and may be long and slender or of a plump oval form. They vary much in size, but seldom exceed 18μ in length.* In fixed and stained preparations they are usually much shorter and more globular than when alive. (Cf. figs. 78 and 80.) The body is usually pointed posteriorly during life, and is compressed laterally, so that its general form is lanceolate or leaf-like. It is usually broadest at the anterior end. At the anterior extremity there is a small snout-like structure, which projects slightly over the small mouth aperture (fig. 78). The contractile vacuole—seen as a clear spot in fig. 78—is very minute, and lies dorsal to the mouth. Food vacuoles, containing ingested bacteria, are present in the protoplasm, chiefly towards the hind end of the body (figs. 79, 80).

The nucleus is more or less central (figs. 78, 79), and has the typical structure. The kinetoplast is oval, and lies at the anterior end—behind, and dorsally to, the mouth. The two flagella are of unequal length, the anteriorly directed one being of about the same length as the body,

* 11-19μ according to Klebs (1892), 8-18μ according to Alexeieff (1911*c*).

while the posteriorly directed one is much longer—often about twice as long. The flagella arise from a pair of tiny blepharoplasts, situated close to the anterior end of the kinetoplast, and lying side by side (fig. 79). In this species the trailing flagellum is often adherent to the body at the anterior end—a point first noted by Dujardin (1841).

The CYSTS of this species are small oval structures, with thin walls (fig. 81). They measure 5-7 μ in length, and contain, as a rule, a single nucleus and kinetoplast. When first formed the remains of the two flagella can usually be made out within them also. Occasionally the nucleus and kinetoplast divide, so that these two structures appear paired inside the cyst. Small deeply stainable granules are also generally present—sometimes in great abundance (cf. fig. 81).

B. caudatus is easily cultivable in many liquid media (hay infusion, etc.) or on agar plates (see p. 167). Like all the other species of the genus which we have studied, it appears to be a strictly aërobic organism. It is also unable to live long in cultures at 37° C. These two facts appear to prove that it cannot live within the human body, and a number of records of this animal—or other species of the genus—found living "parasitically" in man appear to us, consequently, to be erroneous. Abnormal forms—giants, dwarfs, amoeboid forms, etc.—sometimes occur in old cultures.*

(6) *BODO EDAX* Klebs, 1892.

This species of *Bodo* may also occur coprozoically in human faeces; but it is far less common than the preceding, from which it may be distinguished by the following characters:—

The FLAGELLATES (Pl. V, fig. 82) are typically slightly smaller (6-14 μ, when alive), and are of a more regularly oval shape. As a rule the body is not laterally compressed, and bulges on the dorsal (aboral) surface. The flagella are approximately equal in length—the posterior one being sometimes slightly longer—and are both considerably longer than the body. The kinetoplast is massive and often almost spherical. The "snout" is conspicuous. In most other characters *B. edax* closely resembles *B. caudatus*. Its CYSTS are closely similar.

* A process of "fertilization" has been described in "*Prowazekia cruzi*" (? = *B. caudatus*) by Chagas and Torres (1916). But at present there seems to be no good evidence—in this or any other paper—to prove that conjugation occurs in any species of *Bodo*. Cf. also Woodcock (1916).

This species has recently been well redescribed by Kühn (1915), to whose paper the reader may be referred for further details. It should be noted that some of the names given on p. 175 as synonyms of *B. caudatus*, may belong really to *B. edax*. The organism, for example, called "*Prowazekia cruzi*" by Hartmann and Chagas (1910) may perhaps have been the present species—not *B. caudatus*. In most of the published descriptions of the various "species" of *Bodo* (= *Prowazekia*), the characters requisite for accurate specific determination are not sufficiently considered, and the identification of these forms is therefore largely a matter of guesswork.

Possibly other species of *Bodo* also occur in human fæces; but up to the present we have not identified any but the two just described, nor can we find conclusive evidence of the existence of any but these two in the publications of other workers.

(7) *Cercomonas longicauda* Dujardin, 1841.

Synonyms:

? *Cercomonas longicauda* (Dujardin) Stein, 1878.
Cercobodo longicauda (Dujardin) Senn, 1900.
Cercomonas longicauda (Dujardin) Wenyon, 1910.
Cercomonas parva Hartmann & Chagas, 1910.
Cercomonas longicauda (Dujardin) Alexeieff, 1911.

Flagellates belonging to the genus *Cercomonas* Dujardin, 1841, are common in infusions, and occur occasionally in human faeces: but they never live—so far as is known at present—within the human body. Until recently there has been much doubt regarding the interpretation of this generic name, and many of the species are still very difficult to determine exactly.

All species of this genus (cf. Pl. V, figs. 83, 84, 86, 87), are distinguishable by the following characters: The FLAGELLATES are all small, of changeable "amoeboid" form, and possess a single anterior nucleus with a large central karyosome. They possess, in addition, two flagella having a very characteristic arrangement. Both arise from minute blepharoplasts, placed side by side at the anterior pole of the nucleus—the nuclear membrane being drawn out into a conical process at this pole, with the flagella thus arising from its apex. One flagellum is free, and directed forwards. The other is directed

backwards, and adheres for the greater part of its length to the surface of the body—becoming free, as a rule, for only a short distance at the hind end. A kinetoplast is not found in this genus.

Food, consisting chiefly of small bacteria, is ingested in an amoeboid manner by the surface of the body—especially at the posterior end. There is no permanent mouth, and no contractile vacuole has been demonstrated. Ingested food is contained in the usual food vacuoles in the cytoplasm.

MULTIPLICATION takes place by longitudinal fission, in the typical flagellate manner.

The CYSTS are spherical and uninucleate, and contain numerous brightly refractile granules which stain deeply with iron-haematoxylin. They are able to survive desiccation (Wenyon, 1910*a*).

Cercomonads are easily cultivable in many liquid media, such as hay infusion, and on agar plates such as are used for the cultivation of amoebae (see p. 167). Wenyon (1910*a*) specially recommends "hay infusion to which a small quantity of faeces has been added."

Cercomonas longicauda has been specially studied by Wenyon (1910*a*) and Alexeieff (1911*b*). The distinctive characters of this species are the following: Length from about 5 μ to 10 μ—or more, in greatly drawn-out individuals. Anterior flagellum very long (about three times as long as the body). Posterior flagellum much shorter, only slightly exceeding the body in length. Karyosome relatively small. Cysts 4-6 μ in diameter. (See Pl. V, figs. 83-85.)

(8) *CERCOMONAS CRASSICAUDA* Dujardin, 1841 (*emend.*).

This is another very common species of *Cercomonas*, and occurs coprozoically—though in our experience less often than the preceding—in human faeces. It has recently been carefully studied and described (from infusions) by Alexeieff (1911*b*), and may be distinguished from *C. longicauda* by the following characters (see Pl. V, figs. 86-88): Length up to 10-14 μ. The two flagella short, and approximately equal in length, being equal to, or only slightly longer than, the body. Karyosome relatively large. Cysts usually 5-6 μ in diameter.*

* According to Alexeieff (1911*b*) the cysts measure 9-11 μ in diameter, but I have never found such large cysts in my cultures of this species. His figures of the cysts of *C. longicauda*, moreover (Alexeieff (1911*b*), figs. 6, 7, p. 513), are hardly recognizable as those of a *Cercomonas*. (C. D.)

Probably other species of the genus *Cercomonas* may also be found occasionally leading a coprozoic life in human faeces; but up to the present the foregoing are the only ones that we have been able to identify.*

(9) *Copromonas subtilis* Dobell, 1908.

Synonyms :

? *Monas pileatorum* Perty, 1852.
? *Scytomonas pusilla* Stein, 1878.
? *Scytomonas pusilla* (Stein) Klebs, 1892.
Copromonas major Berliner, 1909.
Scytomonas pusilla (Stein) Alexeieff, 1911.
? *Copromonas ruminantium* Woodcock, 1916.
Scytomonas pusilla (Stein) Schüssler, 1917.

Under the above name a coprozoic flagellate was described some years ago by one of us from the faeces of frogs and toads. A closely similar—and probably identical—form occurs very rarely in human faeces, and the organism will therefore be briefly described here.

There is still some doubt as to the correct name of this flagellate. As was pointed out when the generic name *Copromonas* was introduced (Dobell, 1908), the organism called *Scytomonas* by Stein (1878) is possibly the same. But of this flagellate we have only Stein's crude figures—unaccompanied by any proper description—and the identification is there ore very questionable. However, Alexeieff (1911*b*, 1912*b*) and Schüssler (1917) do not hesitate to assign *Copromonas* to the genus *Scytomonas*, though they give no reasons for so doing. In our opinion it is not now possible to ascertain what the organism really was to which Stein gave the name "*Scytomonas pusilla*." If his figures really

* It should be noted that the foregoing descriptions do not agree in some points with those of Woodcock (1916). This worker considers that *C. longicauda* and *C. crassicauda* are the same species—a conclusion with which I can by no means agree. It seems possible that his view is partly due to his having worked with a mixture of species: but from a cytological point of view his figures leave much to be desired, and I am not prepared to identify them precisely. I may also note that Woodcock believes he has observed conjugation in "*C. longicauda*." I have not done so, and consider—from the account published—that there is little or no evidence that conjugation occurs. The phenomenon observed by Woodcock appears rather to be an abortive or regressive fission ; and the ultimate "encystation" appears to be merely the rounding up of the degenerate product. I have observed such phenomena in several other flagellates, and believe they have nothing to do with conjugation properly so-called. (C. D.)

depict the form which we call "*Copromonas subtilis*," then they are incorrect in several details. In our view the genus *Scytomonas* is not now identifiable, and should therefore be abolished. On the other hand, it appears probable that the flagellate which Klebs (1892) called "*Scytomonas pusilla* Stein" was a "*Copromonas*": but how far Klebs was justified in his identification is open to question, and his species was apparently too small (4·8-6μ) to be *C. subtilis*. It seems probable that the organism named *Copromonas major* by Berliner (1909) was really *C. subtilis*, the distinctive features which he described being mostly due —as is evident from the original description of *C. subtilis*, and the more recent work of Schüssler (1917)—to errors of interpretation. The earliest account of a *Copromonas* is possibly that of Perty (1852), whose similar flagellate was named *Monas pileatorum*.

Up to the present we have found this organism in human faeces on only one occasion. The specimen containing it was sent to one of us (C.D.) by Mr. A. G. Thacker, and was obtained from a military patient in the Kitchener Hospital, Brighton. The flagellates were easily cultivated on agar plates (see p. 167), and numerous cultures were made and carefully studied. The most careful examination of living and fixed and stained specimens has failed to reveal any constant structural character* which enables us to distinguish this form from that occurring in the faeces of frogs: but it should be mentioned that in old cultures a number of very minute individuals made their appearance—a point first observed by Mr. Thacker. These very small individuals were never seen in the original cultures of *C. subtilis* from frogs. It is possible—but we think improbable—that they belong to a distinct species.

Copromonas subtilis (Pl. V, fig. 91) is an oval, uniflagellate organism, of relatively simple structure. Its length ranges from about 7 μ to 20 μ, averaging usually about 15 μ: but the smallest forms, observed in cultures, may measure as little as 4-5 μ (fig. 92). The body is subject to little or no change of shape during life: and this is correlated with the fact that the whole organism is invested with a relatively thick and rigid pellicle. At the more pointed anterior end there is a small subterminal aperture—the mouth—through which solid food is ingested.

* In the majority of the individuals cultivated from human faeces the nucleus appears to lie slightly nearer to the anterior extremity than it does in specimens from frog faeces. This character, however, is not constantly visible. (C. D.)

Extending backwards from the mouth, usually for rather more than half the length of the body, and in a slightly spiral direction, is a long narrow gullet. The posterior half of the body, which is rounded terminally, usually contains conspicuous food-vacuoles, charged, for the most part, with bacteria.

The single flagellum, whose length is rather greater than that of the body, arises at the anterior extremity. It is fairly thick, and as it moves as a rule but slowly—its lashing being particularly noticeable at its free end—it is easily visible during life. The flagellum arises from a minute blepharoplast situated in the wall of the gullet, and in close relation to another structure—the reservoir—at the anterior end of the organism. This reservoir is a clear vesicle, easily visible in the living animal. It is not contractile, but has at its base a very small pulsating vacuole which discharges its contents rhythmically into it.

The nucleus is single and vesicular, more or less centrally placed, and contains a large central karyosome (fig. 91). It is bounded by a delicate nuclear membrane, between which and the karyosome there is a clear zone containing "achromatic" granules and crossed by radial "linin" threads. There is no structure (rhizoplast) uniting the blepharoplast to the nucleus, and no true centriole or centrosome is demonstrable (contrary to the assertions of Berliner, 1909).

MULTIPLICATION takes place by simple longitudinal fission into two, the splitting beginning at the anterior end, and passing gradually backwards. (See Pl. V, fig. 93.) During this process the original flagellum is drawn in; the blepharoplast then divides into two; and finally the new flagella arise by outgrowth from the daughter blepharoplasts. The nucleus divides by amitosis or a simple form of mitosis—the finer details being difficult to make out.

This flagellate is one of the few in which CONJUGATION has been shown to occur (Dobell, 1908). Two individuals approach one another and become united at their anterior ends (fig. 94); the union gradually extending backwards until the organisms are completely fused. "Reduction" divisions of the nuclei occur during this process, and the "reduced" nuclei finally fuse to form a single zygote nucleus. The flagellum of one individual is drawn in during the act of fusion (fig. 94), but the conjoined individuals continue to move actively throughout by the aid of the one which persists. When fusion is complete, the zygote may either become wholly remodelled into a single large flagellate—

which continues to lead an active life, and ultimately divides—or it may encyst. Encystation also appears to take place without previous conjugation.

The CYSTS (Pl. V, fig. 95) are oval or rounded structures, with thin walls and clear contents. They contain a single nucleus, and measure about 7-8 μ in diameter. On hatching, each cyst probably liberates a single small monad.

For further details the reader may be referred to the accounts already published—especially to the original description of the organism (Dobell, 1908).

(10) *HELKESIMASTIX FAECICOLA* Woodcock & Lapage, 1915.

We refer to this species a minute coprozoic flagellate which we have so far cultivated from only a single sample of human faeces. The organism was found in a stale stool, several days old, and proved to be cultivable on agar, on which it grew very rapidly. It is closely similar to the form described from goat's faeces by Woodcock and Lapage (1915).

The FLAGELLATE (Pl. V, figs. 89, 90) is closely similar to a *Cercomonas*, but differs in possessing no anterior flagellum. There is only one flagellum, rooted at the anterior extremity, but which is directed backwards and adheres to the surface of the body, becoming free at the posterior end. The nucleus is vesicular, with a central karyosome, and lies at the anterior extremity. A minute contractile vacuole is present in the middle or hinder part of the body.

The organism is usually more or less oval in shape, but somewhat changeable—like a *Cercomonas* : but its anterior extremity, in front of the nucleus, is usually rigid and pointed. During movement this end is always in advance, and the flagellum is trailed behind. The organism is very small, measuring usually only about 4-6 μ in length. The length of the flagellum—from the point of origin, at the anterior end, to its free tip—is about twice (according to Woodcock and Lapage $2\frac{1}{2}$ to 3 times) that of the body. The flagellum appears to be attached to the nucleus as in *Cercomonas*, but the exact insertion is very difficult to make out, owing to the very small size of all the parts.

MULTIPLICATION is effected by simple longitudinal fission, but we have not been able, as yet, to make out the finer details. Moreover we

have not been able to identify the CYSTS of this flagellate with certainty : but according to Woodcock and Lapage (1915) they are spherical, uninucleate, and measure from 3 μ to 3·5 μ in diameter.

The specimens found in human faeces, and those cultivated on agar, live on bacteria. They ingest these in the hinder region of the body—in the same way as a *Cercomonas:* and the ingested organisms are easily seen in stained preparations (cf. figs. 89, 90). Woodcock and Lapage, however, believed that their organisms did not take up solid food. The dimensions of their flagellates, also, appear to be slightly greater (6-7 μ) than those of ours, and they observed a process of "conjugation" which we have not encountered.* It is possible that the form from human faeces belongs to a different species, but this seems unlikely. Unfortunately Woodcock and Lapage have not published a full description of the cytological characters of their organism, their account being based chiefly upon a study of living specimens, in which it is impossible to make out all the details.

(11) "*COPROMASTIX PROWAZEKI*" Aragão, 1916.

? Synonym :

Tetratricomastix intestinalis Sangiorgi, 1917.

Under the above name a tetramastigine flagellate has recently been described by Aragão (1916, 1916*a*). It was obtained in cultures, made with egg-albumin (0·5 per cent.), from the faeces of a human being and a frog :† and it is evident, from the description, that the organism is a coprozoic form and not an inhabitant of the human body.

The FLAGELLATE, 6-18 μ in length, is described as sub-triangular in outline, and much attenuated at the pointed posterior end. At the flattened anterior end four flagella, of equal length, arise from (?) a single blepharoplast. The nucleus is vesicular, with a large central karyosome, and lies near the anterior end. There is a short rhizoplast, attached to the blepharoplast but not to the nucleus. A mouth,

* The interpretation of the phenomena observed appears to be questionable. Cf. what has been said concerning the "conjugation" of *Cercomonas*, p. 180 footnote, *supra.*

† Up to the present I have never encountered this organism in cultures of human faeces, nor in any of the very numerous cultures (egg-albumin and other media) which I have made from the faeces of frogs and toads. (C. D.)

in the form of a short and straight cleft, is present at the anterior end; but it is stated that there are neither food vacuoles nor a contractile vacuole in the cytoplasm. A few dividing individuals have been described and figured (Aragão, 1916*a*) but no cysts have yet been discovered.

It appears almost certain, from the descriptions and figures, that this organism really belongs to the genus *Tetramitus* Perty, 1852 : but the previously described species of this genus require further investigation. Consequently, while it is probable that "*Copromastix*" is a synonym of *Tetramitus*, it is doubtful whether it belongs to any of the species already known. Certain of Aragão's observations, moreover, appear to be open to question. It would be remarkable, for example, if his flagellate really possesses no contractile vacuole, and it is difficult to believe that it can really possess a mouth but no food vacuoles.

Aragão found "*Copromastix*" in cultures of the faeces of only a single human being, in Brazil; and Leger (1918) states that he has also once observed it in Guiana. No other workers appear to have studied this organism, and further investigation of it—and, indeed, of all the species of *Tetramitus*—is needed before a satisfactory classification of these flagellates can be attempted.

An organism which appears to be, similarly, some species of *Tetramitus*, has also been recently cultivated from human faeces by Sangiorgi (1917). To this organism, which is possibly identical with "*Copromastix*," he has given the name "*Tetratricomastix intestinalis*." It is, at all events, probably a coprozoic species of *Tetramitus* and not an intestinal flagellate; but from the published description it is impossible to identify it more certainly.

(12) "*Toxobodo intestinalis*" Sangiorgi, 1917.

Sangiorgi (1917), in Italy, has recently described a "new" "intestinal" flagellate from man, and given it the above name.

The organism in question was cultivated from human faeces, and is almost certainly a coprozoic form, and not an intestinal inhabitant. From the incomplete description published, it appears probable that it is really a *Spiromonas* Perty, 1852.* Coprozoic species of this genus—

* Cf. also Saville Kent (1880), Woodcock (1916).

from goat dung—have recently been studied by Woodcock (1916). The flagellates are small, elongate, and more or less spirally twisted ("crescentic," according to Sangiorgi), and possess two free flagella—both inserted at the anterior end, one recurrent, the other directed forwards. The nucleus is central, and no kinetoplast is present.

We have not been able to study "*Toxobodo*" ourselves, and merely note the foregoing points in order to call attention to the probability that *Spiromonas* occurs in human faeces. We may also note that another flagellate, recently found in the dung of a horse and the excrement of a tortoise by Alexeieff (1918), and by him named *Alphamonas coprocola*, probably belongs to the same genus. All these organisms require further investigation.

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INDEX.

PLATE II.

All drawings were made from fixed and stained specimens. Magnification 2,000 diameters throughout. Fixation, sublimate-alcohol—unless otherwise stated: staining as indicated. (After Dobell (1919 *a*), but slightly reduced.)

Figs. 1—16. *Entamoeba histolytica.*

Fig. 1. Active large form, containing 3 red blood-corpuscles. From stool of a case of amoebic dysentery. (Stained Weigert's iron-haematoxylin and eosin.)

Figs. 2—7. Successive stages in division. From specimens in sections of ulcers in large intestine of experimentally infected kitten. (Fixed in Bouin's fluid, and stained in various ways.)

Figs. 8, 9. Precystic amoebae, belonging to strains forming large and small cysts respectively. (Fig. 8, Mann's stain: fig. 9, haemalum.)

Figs. 10, 11, 12. Uninucleate, binucleate, and quadrinucleate cysts respectively: from same case as fig. 8. Strain forming cysts with mean diameter of 13·5 μ. (Mann's stain.)

Fig. 13. Quadrinucleate cyst belonging to a strain with cysts measuring 15 μ in average diameter. (Haemalum.)

Figs. 14, 15, 16. Uninucleate, binucleate, and quadrinucleate cysts respectively—belonging to a strain producing cysts with an average diameter of 6·6 μ. (Haemalum.)

Figs. 17—26. *Entamoeba coli.*

Fig. 17. Large active amoeba, from human stool. (Heidenhain's iron-haematoxylin and eosin.)

Fig. 18. Precystic amoeba. Note small size, and freedom from food inclusions. (Mann's stain.)

Figs. 19—22. Successive stages in development of cysts, which contain 1, 2, 4, and 8 nuclei respectively. (Figs. 19, 20, Mann's stain; fig. 21, Bouin's fluid and alcoholic ferric-chloride iron-haematein; fig. 22, Heidenhain's iron-haematoxylin.)

Fig. 23. Very small 8-nucleate cyst of *E. coli*. (Haemalum and eosin.)

Fig. 24. 8-nucleate cyst, containing filamentar chromatoid bodies. (Haemalum.)

Fig. 25. 8-nucleate cyst, containing a sheaf of spicular chromatoids. (Haemalum.)

Fig. 26. Very large cyst, containing 16 nuclei. (Haemalum.)

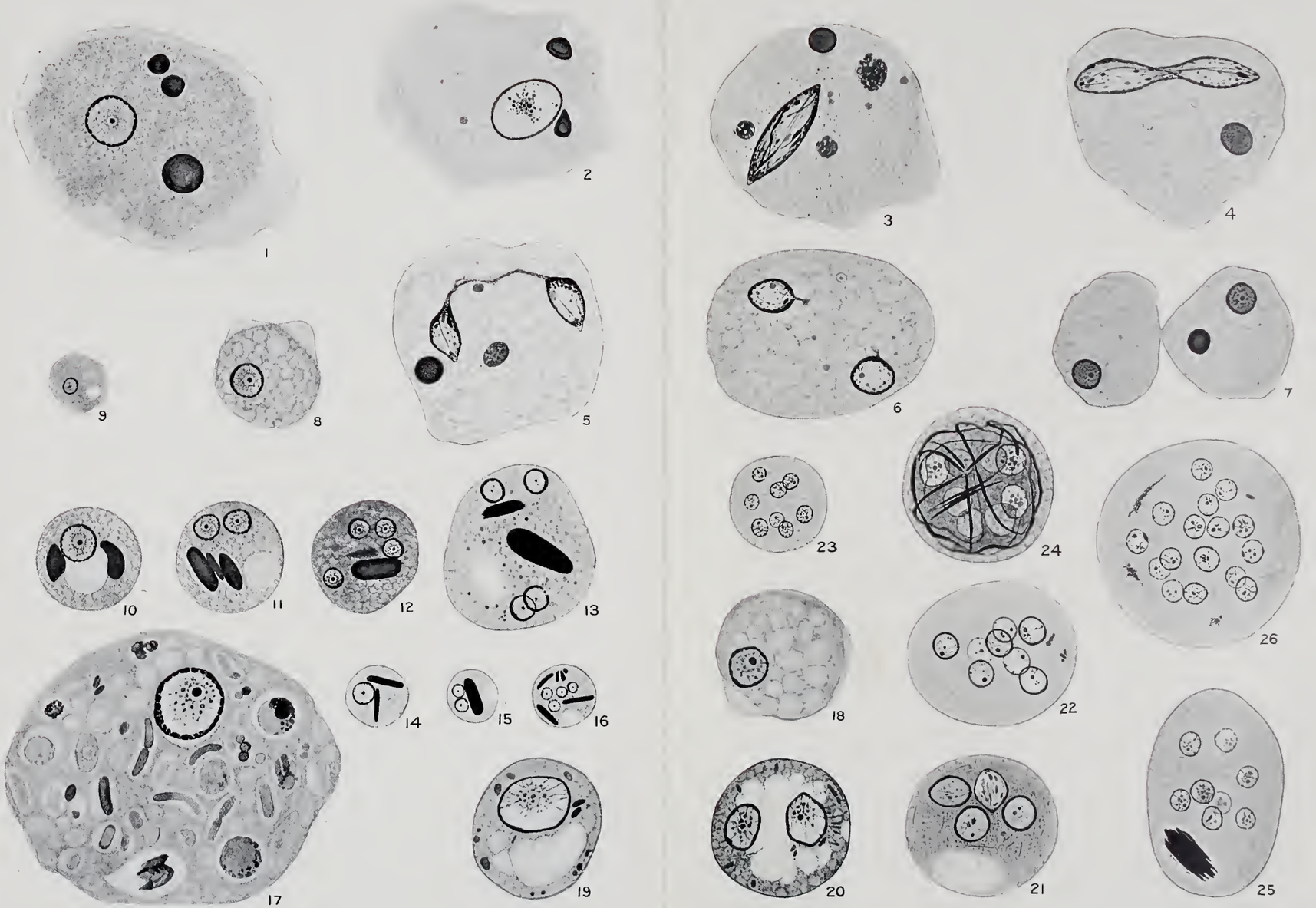


PLATE III.

The left half of the Plate (Fig. 27) illustrates the microscopic appearance of the lesions in Amoebiasis (*E. histolytica* infection).

The right half (Fig. 28) illustrates the size-relations of the cysts of *E. histolytica* belonging to four different races.

Fig. 27. (A) Section of an early intestinal ulcer, with the amoebae in and upon the mucous membrane, which they have partly destroyed. Magnification 90 diameters.

(B) Section of a later and deeper ulcer, showing the amoebae invading the submucous tissue. Magnification 90 diameters.

(C) More highly magnified portion of the base of the ulcer shown in (B). The amoebae are here seen in contact with the healthy submucous tissue—with the destroyed tissue in the cavity of the ulcer in their train, above and to the right. Magnification 450 diameters.

(D) Part of a section through the periphery of an amoebic liver-abscess. Above, healthy liver tissue: below, and in contact with it, amoebae and necrotic tissue in the abscess cavity. Magnification 450 diameters.

All these figures are drawn from sections of experimentally produced lesions in kittens. The material was fixed in Bouin's fluid: figs. (A) and (D) stained with acid fuchsin and picro-indigo-carmine, figs. (B) and (C) Mann's stain.

[From *The Practice of Medicine in the Tropics.*]

Fig. 28. These drawings illustrate the differences in the dimensions of the cysts of *E. histolytica* —belonging to four different strains of the parasite—from four different human infections (Cases H. 8, H. 7, E. 42, B. 1). They show in parallel columns ten cysts from each of these cases—taken at random from fixed and stained preparations, and outlined with the camera lucida. The drawings were made at a magnification of 2,500 diameters, and have been reduced to the size here shown in the process of reproduction. (Only the outlines of the cysts and nuclei are shown, and their chromatoid bodies—in black—when present.)

The remaining figures are outlines, drawn in the same way, and to the same scale, of fixed and stained amoebae of *E. histolytica*. *E. h.* (1), two individuals (containing red corpuscles) from a case of acute amoebic dysentery—belonging to a strain forming cysts similar in size to those of Case E. 42. Fig. *E. h.* (2), two precystic amoebae belonging to a similar strain. Fig. *E. h.* (3), precystic amoebae belonging to a strain with cysts similar in size to those of Case H. 8.

[After Dobell and Jepps (1918).]

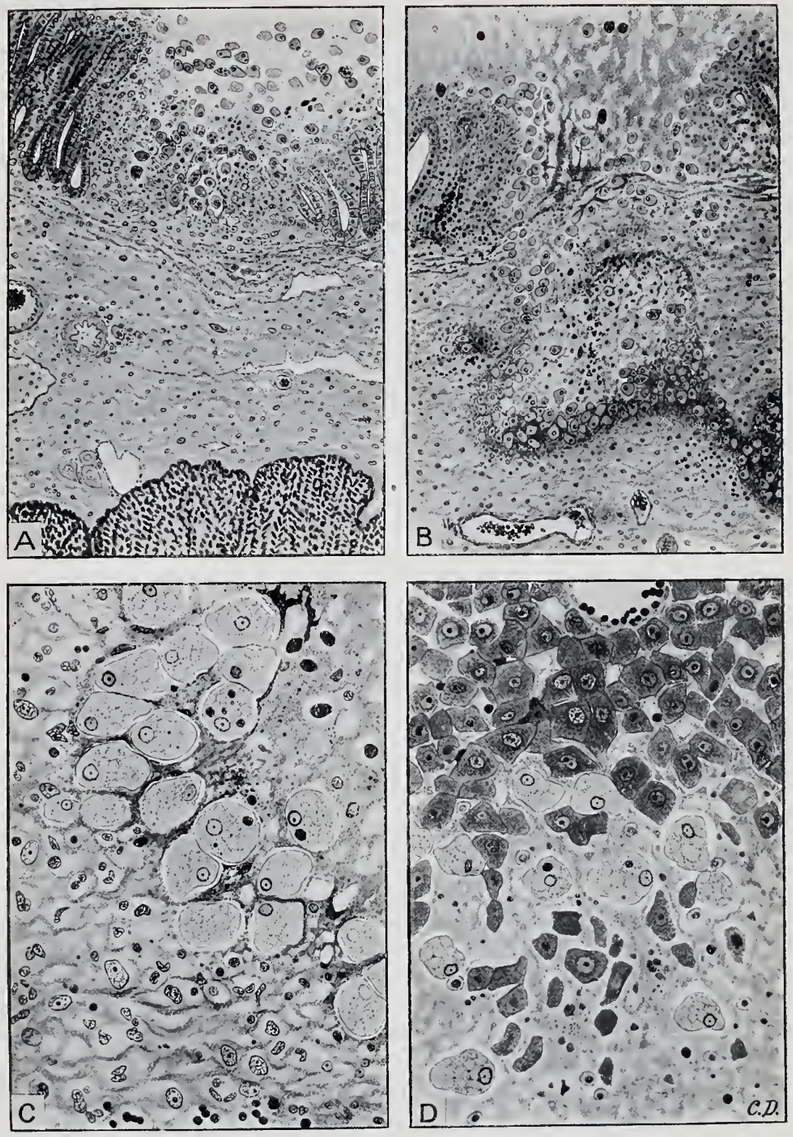


FIG. 27.

FIG. 28.

PLATE IV.

All figures, unless otherwise indicated, drawn from specimens fixed with sublimate-alcohol and stained with Heidenhain's iron-haematoxylin and eosin. Magnification 2,000 diameters throughout.

Figs. 29—48. INTESTINAL AMOEBAE.

Figs. 29—39. *Endolimax nana.*

Figs. 29—32. Four ordinary individuals, showing various common types of nuclear structure.

Figs. 33, 34. Two individuals parasitized by *Sphaerita.* (Fig. 34 stained haemalum.)

Figs. 35, 36, 37. Three successive stages in development of cysts—containing 1, 2, and 4 nuclei respectively.

Fig. 38. Mature 4-nucleate cyst containing filamentar and granular inclusions.

Fig. 39. Supernucleate cyst, containing 8 nuclei. (Haemalum.)

Figs. 40—42. *Dientamoeba fragilis.*

Figs. 40, 41. Two ordinary binucleate individuals.

Fig. 42. A uninucleate specimen.

Figs. 43—48. *Iodamoeba bütschlii.*

Figs. 43, 44. Two ordinary amoeboid individuals.

Fig. 45. Precystic amoeba.

Fig. 46. An organism just encysting.

Figs. 47, 48. Typical cysts—fig. 48 a very irregular specimen, such as is commonly seen in this species. (Haemalum and eosin.)

Figs. 49—57. COPROZOIC AMOEBAE, FROM HUMAN FAECES.

Fig. 49. *Dimastigamoeba gruberi*, amoeboid form.

Fig. 50. *D. gruberi*, free-swimming flagellate form.

Fig. 51. Stage in division (equatorial plate) of amoeboid form of *D. gruberi.*

Fig. 52. Cyst of *D. gruberi.*

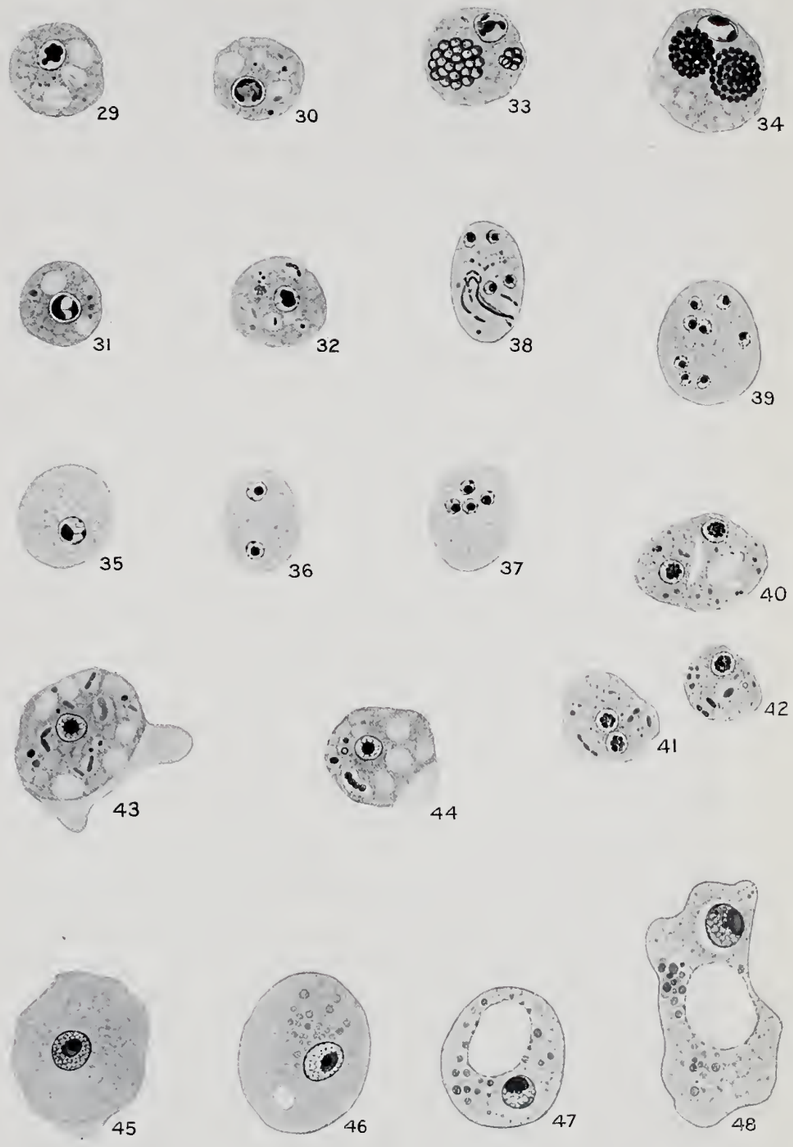
Fig. 53. *Hartmannella hyalina*, ordinary amoeba.

Fig. 54. Stage in division (equatorial plate) of *H. hyalina.*

Fig. 55. Cyst of *H. hyalina.*

Fig. 56. *Sappinia diploidea*, ordinary individual. (Note the two large nuclei, in apposition.)

Fig. 57. Newly formed cyst of *S. diploidea*, containing two individuals.



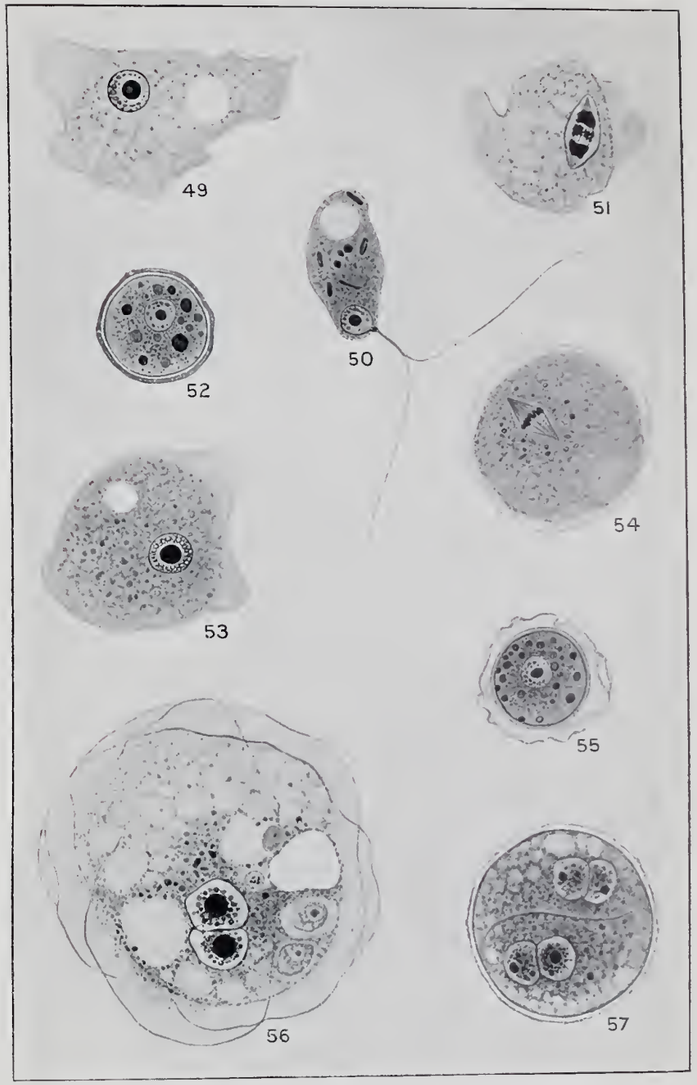


PLATE V.

All drawings made from fixed and stained specimens, unless otherwise indicated. Fixation, sublimate-alcohol: staining, Heidenhain's iron-haematoxylin, usually combined with eosin. Magnification 2,000 diameters throughout.

Figs. 58—77. INTESTINAL FLAGELLATES.

Figs. 58—61. *Giardia intestinalis.* (Fixation: Bouin's fluid.)

Fig. 58. Active flagellate, ventral view.

Fig. 59. Similar flagellate, in profile (ventral surface to right, dorsal to left of figure).

Fig. 60. Binucleate cyst.

Fig. 61. Quadrinucleate cyst—later stage of development.

Figs. 62—68. *Enteromonas hominis.*

Fig. 62. Active flagellate; typical form, with 4 flagella—3 free, and 1 recurrent and adherent to the body ("*Tricercomonas*" of Wenyon and O'Connor).

Fig. 63. Form in which the recurrent flagellum is not clearly visible ("*Enteromonas*" of Fonseca).

Fig. 64. Form in which only 2 anterior flagella are visible ("*Diplocercomonas*" of Chalmers and Pekkola).

Fig. 65. Typical form, showing 2 blepharoplasts.

Figs. 66—68. Uninucleate, binucleate, and quadrinucleate (mature) cysts, respectively.

Figs. 69—71. *Trichomonas hominis.*

Fig. 69. Small individual, with 3 anterior flagella.

Fig. 70. Large individual, 3 anterior flagella ("*Tritrichomonas*").

Fig. 71. Individual with 4 anterior flagella ("*Tetratrichomonas*").

Figs. 72, 73. *Embadomonas intestinalis.*

Fig. 72. Active flagellate.

Fig. 73. Cyst.

Figs. 74—77. *Chilomastix mesnili.*

Fig. 74. Active flagellate, ventral view.

Fig. 75. Smaller individual, from right side.

Fig. 76. Individual seen antero-ventrally—to show the arrangement of blepharoplasts and organs arising from them.

Fig. 77. Mature cyst. (Fixation: Bouin's fluid.)

Figs. 78—95. COPROZOIC FLAGELLATES FROM HUMAN FAECES.

Figs. 78—81. *Bodo caudatus.*

Fig. 78. Living organism—unstained.

Figs. 79, 80. Stained specimens. (Fixation: alcoholic picro-acetic.)

Fig. 81. Cyst, stained specimen.

Fig. 82. *Bodo edax*, active flagellate.

Figs. 83—85. *Cercomonas longicauda.*

Fig. 83. Living flagellate, creeping. Unstained.

Fig. 84. Stained specimen.

Fig. 85. Cyst—living and unstained.

Figs. 86—88. *Cercomonas crassicauda.*

Figs. 86, 87. Active flagellates (stained alcoholic iron-haematein).

Fig. 88. Cyst (stained as preceding).

Figs. 89, 90. *Helkesimastix faecicola*, 2 flagellates.

Figs. 91—95. *Copromonas subtilis.*

Fig. 91. Ordinary flagellate.

Fig. 92. Dwarf form, from culture.

Fig. 93. Stage in longitudinal division.

Fig. 94. Early stage of conjugation.

Fig. 95. Cyst.

Fig. 96. *Chlamydophrys stercorea.*

An individual from the faeces of a toad. (The filose pseudopodia projecting through the shell opening are contracted as a result of fixation with formalin.)

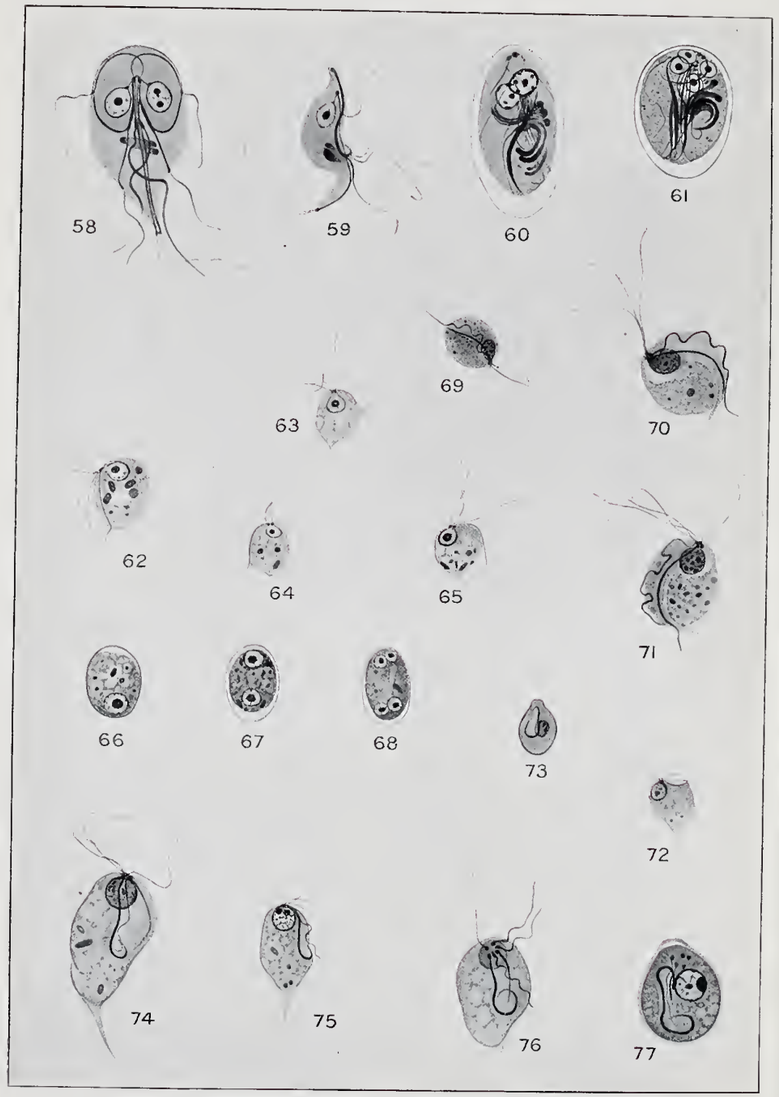


PLATE V.

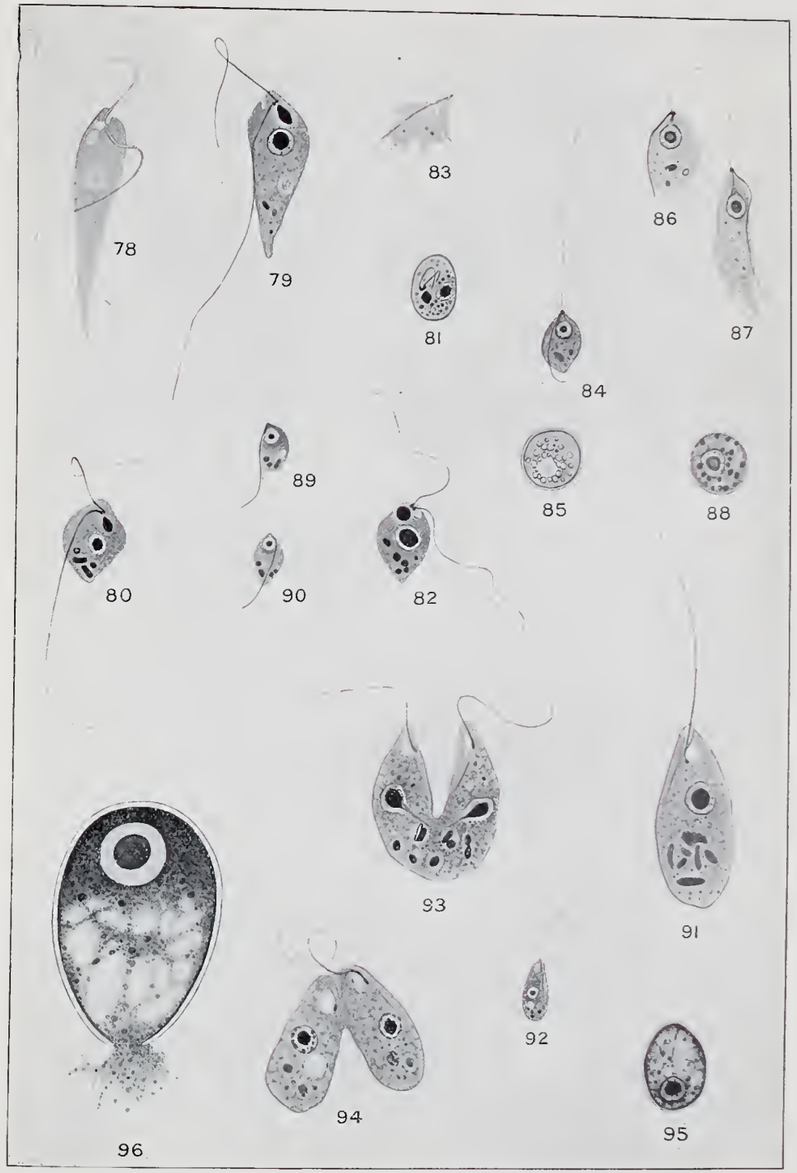


PLATE VI.

All drawings represent living and unstained specimens. Magnification 2,000 diameters throughout.

Figs. 97—102. *Isospora hominis.*

Fig. 97. Oöcyst with unsegmented protoplasm, as usually passed in stools.

Fig. 98. Later stage; nucleus divided into two.

Fig. 99. Later stage; protoplasm segmented into two sporoblasts.

Fig. 100. Fully developed oöcyst, containing two spores—each containing four sporozoites.

Figs. 101, 102. Degenerate oöcysts, which have failed to develop.

Fig. 103. *Eimeria oxyspora.*

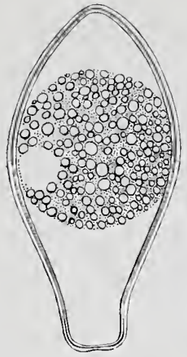
A ripe oöcyst, containing four fully-formed spores.

Fig. 104. *Eimeria wenyoni.*

A ripe oöcyst, containing four fully-formed spores. (After Wenyon, 1915.)

Fig. 105. *Eimeria snijdersi.*

Ripe oöcyst, with four fully-developed spores. (Combined from figures and specimens of Dr. E. P. Snijders.)



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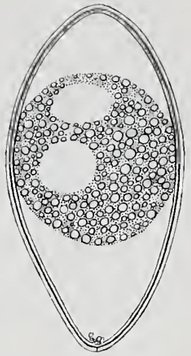

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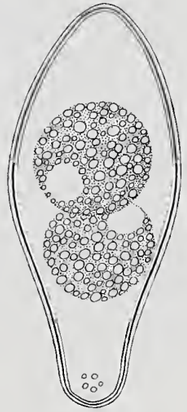

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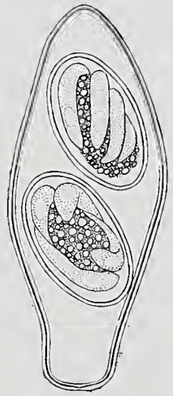

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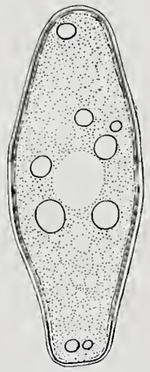

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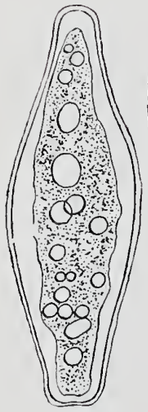

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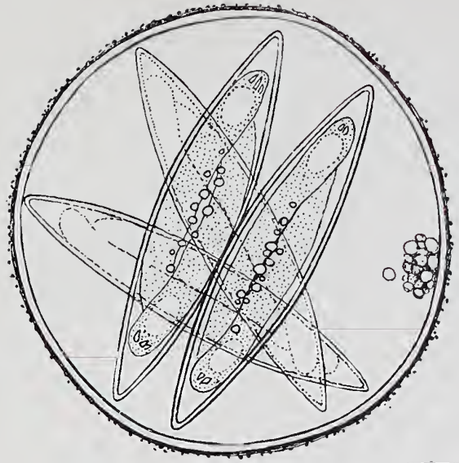

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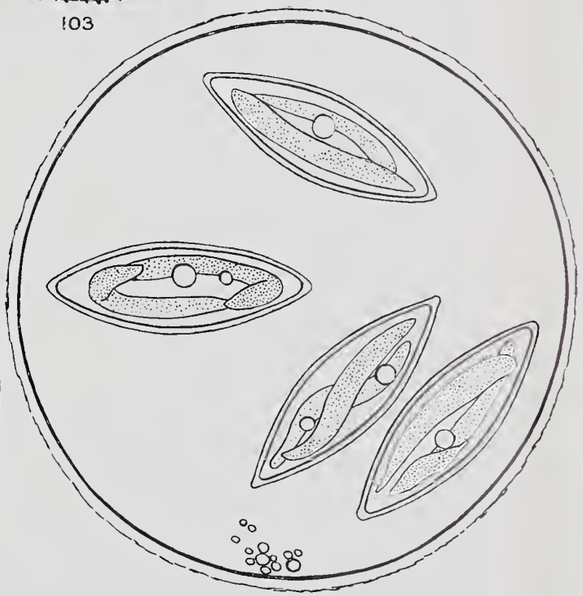

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PLATE VII.

Fig. 106. *Balantidium coli.* Active ciliate, semidiagrammatic. Living specimen, seen from left side.
N. = meganucleus.
n. = micronucleus.
c.v.1 = anterior contractile vacuole.
c.v.2 = posterior contractile vacuole.
f.v. = food vacuole.
mo. = mouth.
Magnification 2,000 diameters. (The sketch was made from an individual in the faeces of a pig.)

Fig. 107. *Balantidium minutum.* × 2,000. (Drawing made from Schaudinn's figures and description.) Ventral view.

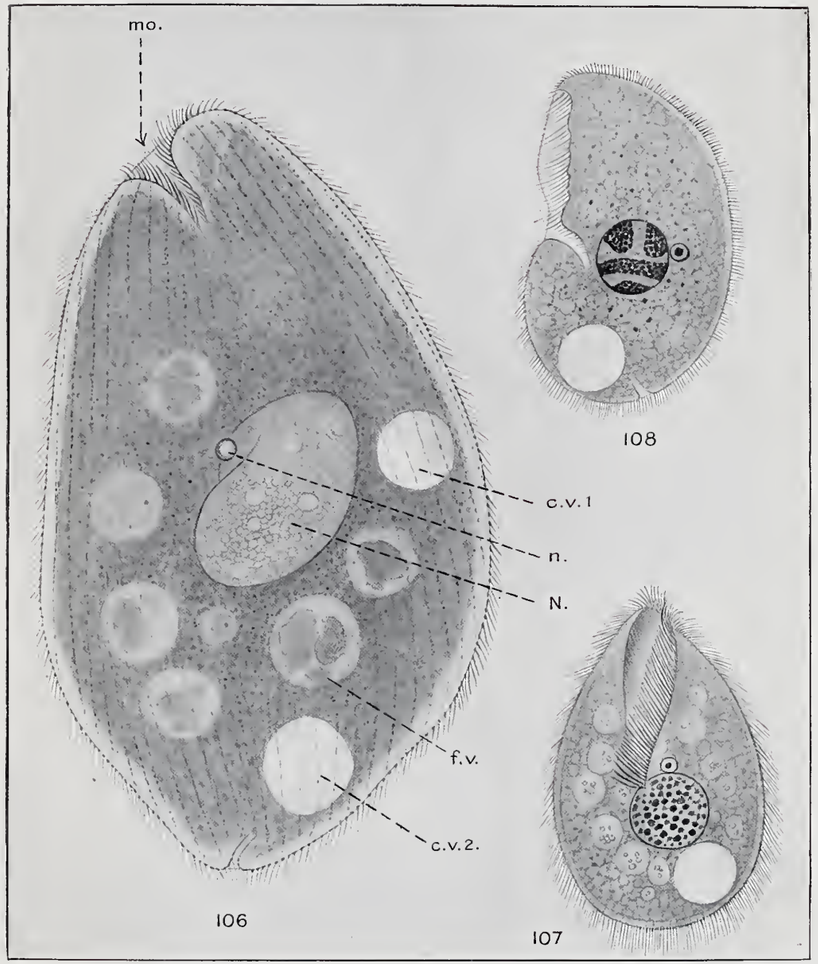
Fig. 108. *Nyctotherus faba.* × 2,000. (Drawing made from Schaudinn's figures and description.) From left side.

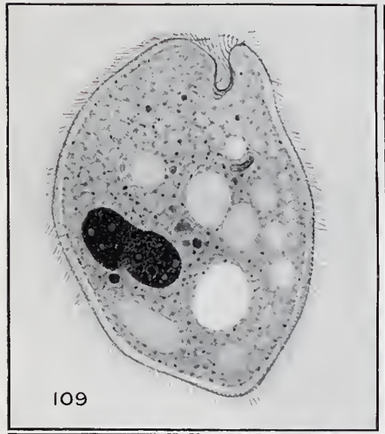
Fig. 109. *Balantidium coli.* × 1,000. Specimen from stool of a human case of Balantidiosis. Fixed sublimate-alcohol, stained Heidenhain's iron-haematoxylin.

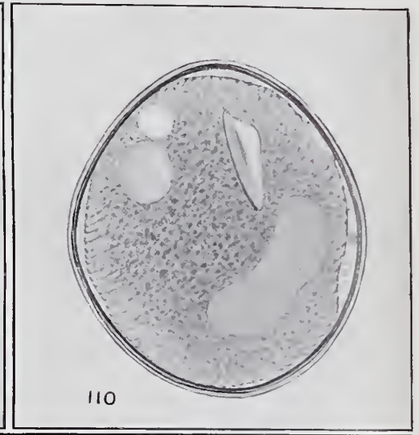
Fig. 110. *Balantidium coli.* Cyst, × 1,000. Living; from faeces of pig.

Fig. 111. Part of the periphery of a Balantidial Ulcer: colon of human case of balantidiosis. × 50. (Section stained iron-haematoxylin and orange G.) Above and to the right, the cavity of the ulcer, filled with necrotic tissue: below and to the left, numerous balantidia in the submucous tissue.

[Figs. 109-111 from *The Practice of Medicine in the Tropics.*]







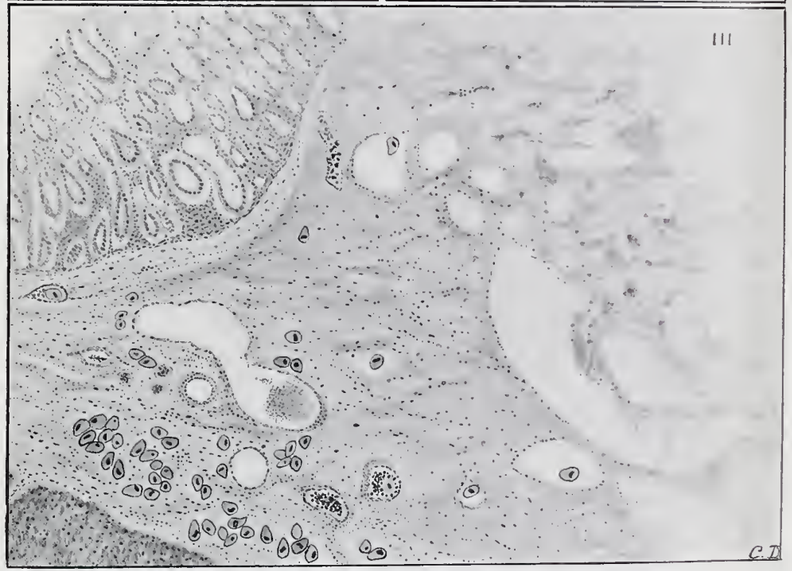


PLATE VIII.

Semi-diagrammatic figures of the CYSTS OF THE CHIEF INTESTINAL PROTOZOA OF MAN. These figures have been made as aids to diagnosis. They are not drawn from actual specimens, but are not "diagrammatic" in the sense that they are unlike the objects which they are intended to depict. On the contrary, they have been drawn to look as much like the actual objects as possible. (Cf. Preface, p. vii.) Magnification 2,000 diameters throughout.

THE LEFT-HAND PANEL of the Plate shows the cysts as they appear when ALIVE AND UNSTAINED.

THE MIDDLE PANEL shows THE SAME CYSTS as they would appear when mounted and examined IN IODINE SOLUTION.

The RIGHT-HAND PANEL shows THE SAME CYSTS as they would appear when FIXED AND STAINED WITH IRON-HAEMATOXYLIN.

Each cyst is labelled with the same letter throughout, but is distinguished by a different index number (1, 2, or 3) on each panel of the plate. Fig. A, for example, is marked A^1, A^2, A^3, according as it represents the same cyst alive (A^1), in iodine (A^2), or after fixation and staining (A^3). The cysts are shown lying in the SAME POSITION in each figure, so that they can be readily compared.

Figs. A, B, C. 1-nucleate, 2-nucleate, and 4-nucleate cysts respectively of *Entamoeba histolytica* —a strain with cysts ca. 12μ in diameter.

Figs. D, E. 1-nucleate and 4-nucleate cysts of *E. histolytica* —strain with small cysts, ca. 7·5μ in diameter.

Fig. F. Mature (1-nucleate) cyst of *Iodamoeba bütschlii*.

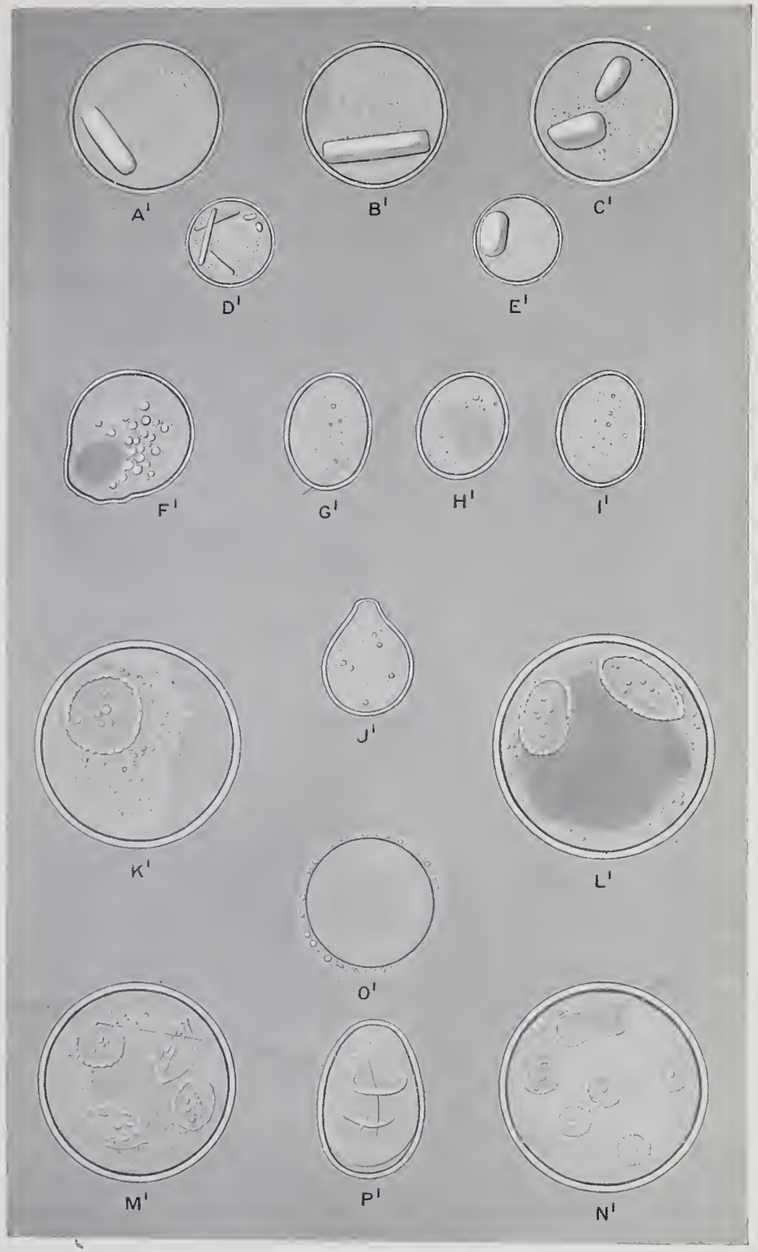
Figs. G, H, I. 1-nucleate, 2-nucleate, and 4-nucleate cysts respectively of *Endolimax nana*. (H is a cyst containing a lump of glycogen.)

Fig. J. Mature (1-nucleate) cyst of *Chilomastix mesnili*.

Figs. K, L, M, N. 1-nucleate, 2-nucleate, 4-nucleate, and 8-nucleate cysts respectively of *Entamoeba coli*.

Fig. O. A specimen of *Blastocystis hominis*—for comparison.

Fig. P. A 4-nucleate cyst of *Giardia intestinalis*.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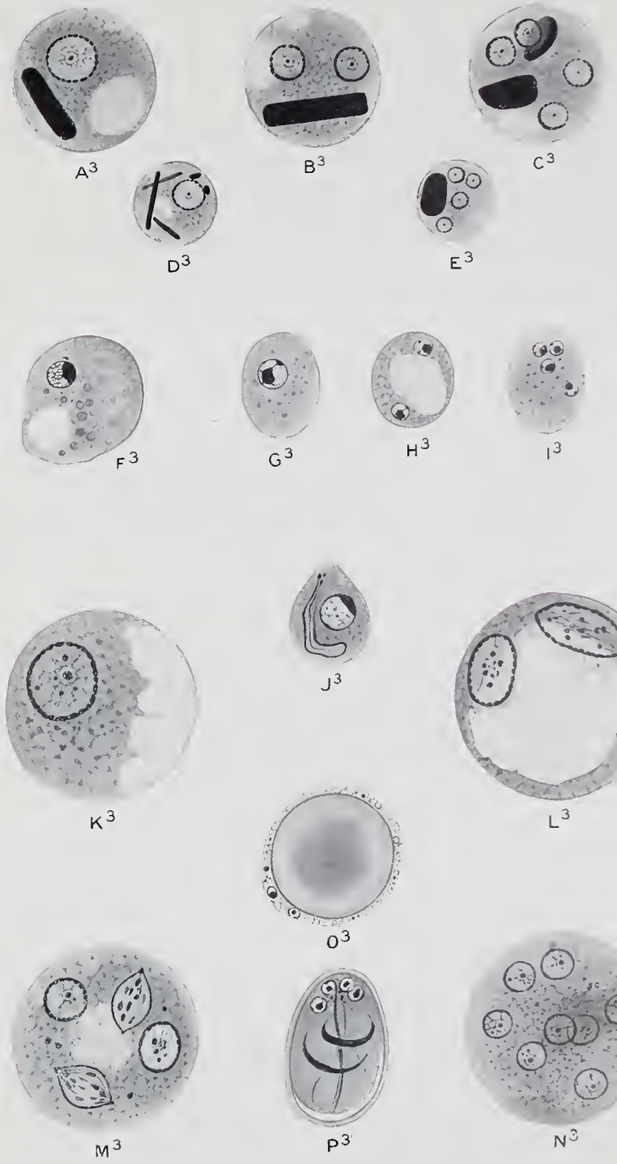
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